

GROWTH AND PHYSIOLOGICAL
RESPONSES OF *EUCALYPTUS*
GLOBULUS LABILLADIÈRE
FOLLOWING DEFOLIATION.

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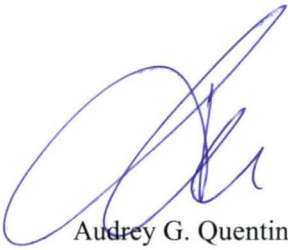
University of Tasmania

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"Few there are [. . .] who seem to clearly realize how broad a lesson on the life-history of plants is written in the trees that make the great forest regions of the world." Clarke 1894

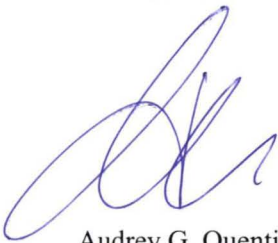
Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

A handwritten signature in blue ink, consisting of a large, stylized 'A' followed by 'G. Quentin'.

Audrey G. Quentin

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Abstract

Many defoliating herbivores cause damages to *Eucalyptus globulus* Labill. plantations, reducing the quality and value of the wood products they source. This damage ranges from mild to severe removal of leaf surface, and can result in significant reductions in yield. Our knowledge of physiological responses to defoliation in this species is incomplete, with previous studies focusing on very young trees and ignoring the potential interaction of abiotic stress with defoliation. Projections of future climates in many of the eucalypt-growing parts of the world suggest that abiotic stress, particularly degree of water stress will increase. In addition, there is strong evidence that changing climate, and particularly increasing temperature, is likely to favour many of the defoliating pests commonly associated with *E. globulus*.

The objectives of work described in this thesis were to investigate the physiological strategies adopted by *E. globulus* in response to a defoliation event, determine their effects on growth and water relations, and examine the interaction with limited water supply, with the aim of improving our capacity to predict the impact of defoliation on tree productivity and water use.

The main studies investigating tree responses to defoliation have utilised artificial defoliation treatments rather than natural defoliation because of logistical constraints. Although artificial manipulations are assumed to have significant advantages, the adequacy of the artificial method has been questioned in term of accuracy and differences in the plant responses. I tested that *E. globulus* respond differently to both artificial and natural defoliations. The results showed that the directions of response to artificial and insect defoliation were very similar. However, the influence of differential magnitude of the responses was more difficult to ascertain. I conclude that artificial defoliation may not accurately reflect the full strength of effects from insect defoliation, and caution must be exercised in extrapolating results of simulated herbivory experiments.

Most previous studies of *E. globulus* responses to defoliation have focused on young, pre-canopy closure trees, and none have examined physiological responses of older trees to defoliation. Substantial defoliation can occur post-canopy closure. The effects of a single defoliation event on 4-year-old *E. globulus* were investigated on growth, photosynthetic and water relation responses in non-limited water supply conditions. The trees responded to removal of 45% of leaf area by a transient change in stem growth, change in crown architecture, the up-regulation of photosynthesis likely *via* the improvement of tree water status. It was concluded that 4-year-old *E. globulus* were able to compensate for the loss of foliage.

Plantations of *E. globulus* are being established increasingly on lower rainfall sites, and in addition drought conditions are projected to increase in many areas of Australia over the next century. There is little understanding of the interactions of water stress and defoliation, although some results suggest that defoliation may be beneficial to trees growing under water limitation. I tested the hypothesis that partial defoliation would alleviate the effects of water stress. The effect leaf removal on 75% of crown length of 1-year-old *E. globulus* on growth, gas exchange and water use was examined in irrigated and rain-fed plots. Over a short-term period, trees responded to the interaction of limited water supply and defoliation by maintaining tree growth, increasing tree transpiration rate per unit leaf area, canopy conductance and hydraulic conductance, while maintaining the gradient of leaf water potential constant. It was concluded that defoliated trees were able to ease the effect of water stress by improving plant water status. Also, the findings were meeting the requirements of the theoretical hydraulic model.

Acknowledgments

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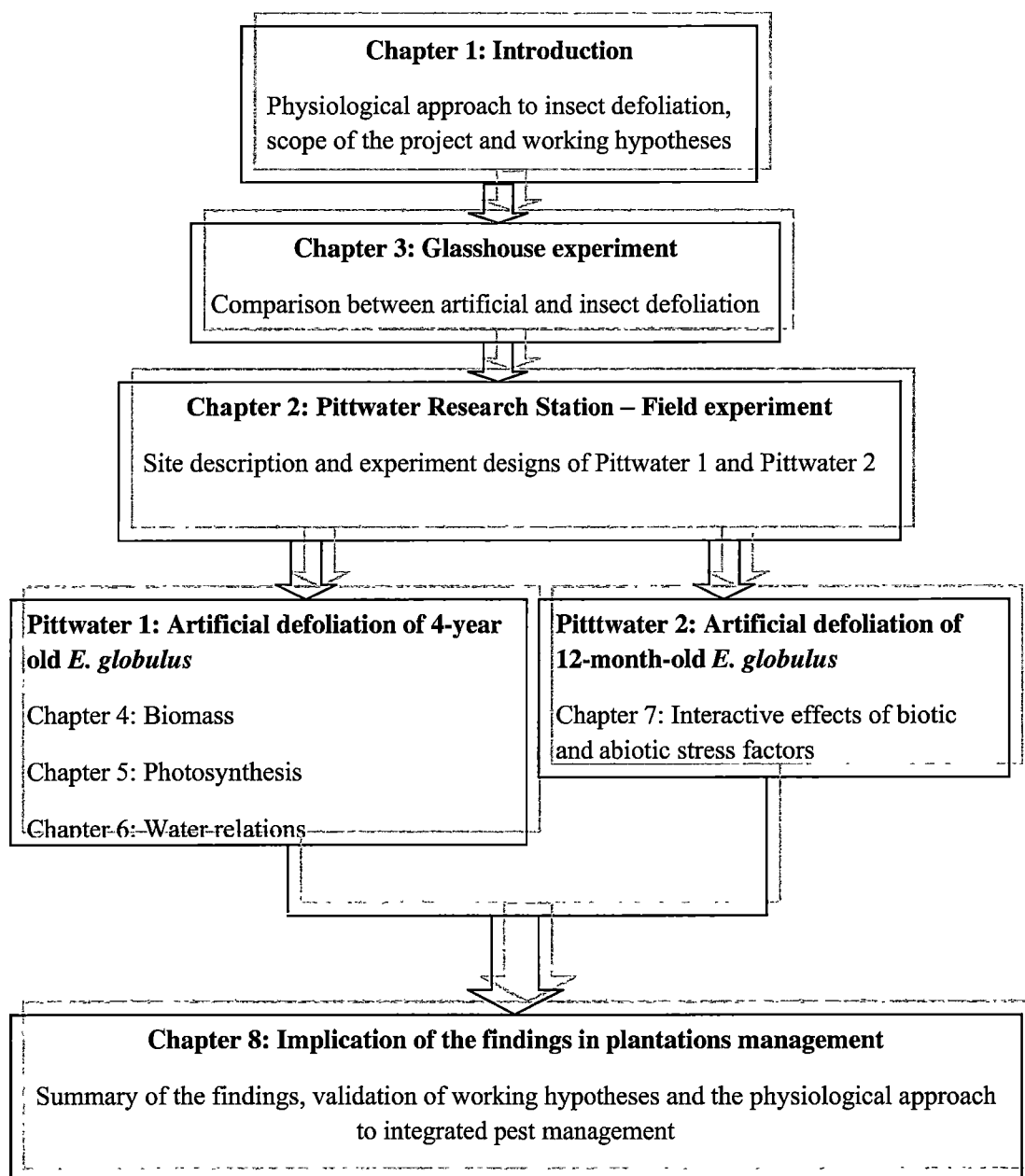
Merci à tous (Thank you all)!

Thesis structure

Each chapter/section of this thesis is a separate piece of work, assesses specific hypotheses and has been written in manuscript format. However to enhance the continuity of the thesis, abstracts have been omitted, symbols and their units have been listed at the beginning of the thesis, references have been collated at the end of the thesis, acknowledgements for each publication have been detailed separately (see below), and figures and tables have been renumbered according to chapter. The manuscripts embedded in this thesis have been reviewed or are under review in the CSIRO internal review process and are either published, accepted or submitted to their respective journals unless otherwise stated.

The thesis is divided into seven chapters. Chapter 1 investigates the current knowledge in insect defoliation and plant response. This chapter also proposes four working hypothesis that current explain plant response to defoliation. Chapter 2 describes the design, establishment, management, maintenance and plant materials of the field experiments at which the researches were conducted. Chapter 3 describes the impacts of both methods on height and diameter increment; above-ground biomass allocation; foliar chemistry; stimulation of photosynthesis of young *E. globulus* seedlings; and discusses the suitability of artificial method to mimic natural defoliation. The experiment was undertaken in controlled glasshouse conditions. Chapter 4 examines how a four-year-old, closed canopy stand of *E. globulus* would respond morphologically to partial leaf removal. Chapter 5 reports a field experiment using 13-meter trees, in which maximum light-saturated CO₂ assimilation was measured for individual leaves of defoliated and non-defoliated (control) trees. The experiment was designed to gain insight into the photosynthetic compensatory response. Measurements were made in three crown zones, over a period of 7 months. Changes in stomatal conductance, foliar nutrient concentrations, and water-use efficiencies were also investigated. Chapter 6 examines changes diurnal patterns of transpiration and canopy conductance in 4-year-old defoliated and non-defoliated *E. globulus* trees growing under similar atmospheric conditions and in an irrigated plot. Climatic data including temperature, vapour pressure deficit and solar radiation were simultaneously recorded over the period of the experiment. Predawn and midday leaf water potential were also measured. Chapter 7 aims at investigating trees responses to

the combined action of these stress factors. This experiment included the application of defoliation subsequent to the low water supply on 12-month-old *E. globulus*. Changes in photosynthesis, transpiration, leaf water potential and stomatal conductance were investigated over a 3-month period. Chapter 8 presents a summary of all the results presented in the preceding chapters and considers them in the context of the implications for forest management.



Manuscripts

The manuscripts embedded in this thesis are as follows:

1. Quentin, A.G. Physiological approach of insect defoliation in evergreen trees. International Forest Review, in preparation.

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2. Quentin, A.G., Pinkard, E.A., Beadle C.L., Wardlaw, T.J., O'Grady, A.P., Paterson S. and Mohammed C.L. (2010) Do artificial and natural defoliation have similar effects on physiology of *Eucalyptus globulus* Labill. seedlings? Annals of Forest Science, 67: 203-211.

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3. Quentin, A.G., Pinkard, E.A., Beadle C.L. and O'Grady, A.P. (2010) Effects of partial defoliation on closed canopy *Eucalyptus globulus* Labill.: Growth, biomass allocation and carbohydrates. Forest, Ecology and Management, under review.

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4. Quentin, A.G., Beadle C.L., O'Grady, A.P., Pinkard, E.A. and Mohammed C.L. (2010) Responses of *Eucalyptus globulus* Labill. to moderate artificial defoliation: traits controlling photosynthesis. Tree Physiology, under review.

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5. Quentin, A.G., O'Grady, A.P., Beadle C.L., Worledge, D. and Pinkard, E.A. (2010) Responses of transpiration and canopy conductance to partial defoliation of *Eucalyptus globulus* trees. Agricultural and Forest Meteorology, under final review.

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6. Quentin, A.G, Beadle C.L., O'Grady, A.P., Pinkard, E.A. and Mohammed C.L. (2010) Interactive effects of water supply and defoliation on *Eucalyptus globulus* photosynthesis, plant water status and growth. Tree Physiology, under review.

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Table of contents

DECLARATIONS..... i

ABSTRACT..... ii

ACNKOWLEDGEMENTSiv

THESIS STRUCUTRE.....vi

MANUSCRIPTS.....ix

TABLE OF CONTENTSxi

LIST OF FIGURES.....xiv

LIST OF TABLES..... xviii

DEFINITION OF SYMBOLSxx

CHAPTER 1. GENERAL INTRODUCTION

1. 1. DEFOLIATING INSECTS: A CHALLENGE FOR CURRENT AND
FUTURE FOREST GROWTH1

1.2. GROWTH RESPONSE TO DEFOLIATION: WHAT DO WE KNOW?2

 1.2.1 *Types of growth response*2

 1.2.2 *Variation in the growth response*5

 1.2.3. *Physiological approach: can it explain the growth response?*6

 1.2.4. *Role of site factors in responses to defoliation*9

1.3. THE PROJECT10

 1.3.1. *Eucalyptus globulus plantations*10

 1.3.2. *Insect defoliation in E. globulus*.....13

 1.3.3. *Scope of the project*13

1.4. HYPOTHESES IN PLANT DEFOLIATION14

CHAPTER 2. DESCRIPTION OF THE PITTWATER EXPERIMENTS

2.1. SITE DESCRIPTION20

2.2. PLANT MATERIALS.....21

 2.2.1. *Experiment 1*21

 2.2.2. *Experiment 2*21

2.3. BIOPHYSICAL ENVIRONMENT.....22

CHAPTER 3. NATURAL AND ARTIFICIAL DEFOLIATIONS

3.1. INTRODUCTION24

3.2. MATERIALS AND METHODS.....26

3.2.1. Conditions of glasshouse	26
3.2.3. Plant material.....	26
3.2.3. Insect culture	27
3.2.4. Treatments and experimental design	27
3.2.5. Stem growth.....	28
3.2.6. Gas exchange and chlorophyll concentration	29
3.2.7. Foliar chemistry	29
3.2.8. Biomass harvesting.....	30
3.2.9. Data analysis	30
3.3. RESULTS.....	31
3.3.1. Growth increment	31
3.3.2. Photosynthesis, stomatal conductance and water-use efficiency.....	31
3.3.3. Foliar chlorophyll concentration and A_{max}	35
3.3.4. Foliar N concentration and photosynthetic nitrogen-use efficiency	35
3.3.5. Foliar non-structural carbohydrate conte	38
3.3.6. Specific leaf area and biomass partitioning	38
3.4. DISCUSSION	41

CHAPTER 4. GROWTH, BIOMASS ALLOCATION AND CARBOHYDRATE

4.1. INTRODUCTION	45
4.2. MATERIELS AND METHODS	47
4.2.1. Defoliation treatment.....	47
4.2.2. Growth responses	48
4.2.3. Biomass harvesting.....	48
4.2.4. Non-structural carbohydrate analysis	50
4.2.5. Data analysis.....	50
4.3. RESULTS.....	52
4.3.1. Stem growth.....	52
4.3.2. Leaf area development.....	52
4.3.3. Branch variables	56
4.3.4. Biomass partitioning and allocation	56
4.3.5. Carbohydrate partitioning and dynamics	60
4.4. DISCUSSION	60

CHAPTER 5. TRAITS CONTROLLING PHOTOSYNTHESIS

5.1. INTRODUCTION	67
5.2. MATERIELS AND METHODS	69
5.2.1. Treatments.....	69
5.2.2. Gas exchange	71
5.2.3. Specific leaf area and chemical analyses	71
5.2.4. Data analysis.....	72
5.3. RESULTS.....	73
5.3.1. Specific leaf area	73
5.3.2. Light-saturated photosynthetic rate and stomatal conductance	76
5.3.3. Relationship between photosynthetic rate and stomatal conductance	76
5.3.4. Foliar nitrogen, phosphorus and carbohydrate concentrations	80

5.3.5. Instantaneous N-use efficiency	80
5.4. DISCUSSION	80

CHAPTER 6. RESPONSE OF TRANSPIRATION AND CANOPY CONDUCTANCE

6.1. INTRODUCTION	85
6.2. MATERIELS AND METHODS	86
6.2.1. Tree water use	86
6.2.2. Canopy conductance.....	87
6.2.3. Leaf water potential.....	88
6.2.4. Soil-to-leaf hydraulic conductance.....	88
6.2.5. Model development.....	88
6.2.6. Data analysis	89
6.3. RESULTS	92
6.3.1. Climate and soil matric potential.....	92
6.3.2. Leaf water potential.....	95
6.3.3. Transpiration and canopy conductance	97
6.2.4. Soil-to Leaf Hydraulic conductance.....	97
6.2.5. Canopy conductance and atmospheric variables relationships.....	98
6.4. DISCUSSION	98

CHAPTER 7. INTERACTIVE EFFECTS OF WATER SUPPLY AND DEFOLIATION

7.1. INTRODUCTION	107
7.2. MATERIELS AND METHODS	110
7.2.1. Experimental design	110
7.2.2. Stem growth.....	111
7.2.3. Gas exchange	111
7.2.4. Leaf water potential.....	112
7.2.5. Whole tree water use	112
7.2.6. Data analysis	113
7.2.7. Modelling analysis with CABALA.....	113
7.3. RESULTS	116
7.3.1. Compensatory responses	116
7.3.2 Regulation of tree's capability for transporting water to the leaves	116
7.3.3. Defoliation offset the negative effects imposed by the rain-fed treatment on growth.....	117
7.3.4. How well does CABALA predict impacts of defoliation on tree response?	124
7.4. DISCUSSION	124

CHAPTER 8. IMPLICATIONS FOR MANAGEMENT131

References.....139

List of figures

Figure 1.1. Type 1 and Type 2 responses to defoliation treatments show distinctive patterns in yield increment.

Figure 1.2. Effects of internal factors on the physiological response of plant to defoliation.

Figure 1.3. Effects of external factors on the physiological response of plant to defoliation.

Figure 3.1. Mean increments of (a) height (h_{inc}) and (b) basal diameter (d_{inc}) measured on four occasions for control, artificial and insect. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 3.2. Mean (a) photosynthetic rate (A_{max}), (b) stomatal conductance (g_s), and (c) water-use efficiency (WUE) for control, artificial and insect defoliation treatments on four occasions after the defoliation. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 3.3. Mean (a) chlorophyll concentration (Chl) and (b) ratio between A_{max} and Chl ($A:Chl$) on four occasions following defoliation treatments. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 3.4. Means (a) nitrogen (N) and (b) phosphorus (P) concentrations 27 days after the defoliation in zones 2 and 3 (Z2, Z3). Error bars show least square differences ($\alpha<0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 3.5. Mean specific leaf area (SLA) for control and defoliated seedlings 27 days after defoliation. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 4.1. Mean (a) height (h_{inc}) and (b) diameter at 1.3 m height (d_{inc}) increment of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on eight occasions after defoliation. Errors bars represent standard errors ($\alpha=0.05$).

Figure 4.2. Defoliation treatment x crown zones interaction effects on (a) specific leaf area (SLA) and (b) leaf area (A_L) of undefoliated and defoliated *E. globulus* trees 322 days after treatment imposed. Values are means of three replicates \pm 95% LSD bars.

Figure 4.3. Defoliation treatment x crown zones interaction effects on the number of (a) dead branches in the lower (Z_L) and middle (Z_M) crown zones, and (b) newly

produced branches in the upper crown zone (Z_U) of undefoliated and defoliated *E. globulus* 322 days after treatment imposed. Values are means of three replicates \pm 95% LSD bars.

Figure 4.4. Mean biomass of whole-tree below-ground (coarse roots) and above-ground organs (stem, branch and foliage) of control and defoliated *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on 322 days after defoliation. Errors bars represent standard errors ($\alpha=0.05$).

Figure 4.5. Mean concentration of (a) soluble sugar, (b) starch and (c) total non-structural carbohydrate of below-ground (coarse roots) and above-ground organs (stem, branch, foliage) of *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on 322 days after defoliation.

Figure 5.1. Illustration representing the delineation of the three crown-zone prior to defoliation.

Figure 5.2. Mean light-saturated photosynthetic rates (A_{\max}) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliated treatments at Pittwater from late winter 2006 to autumn 2007. Error bars show mean standard errors ($\alpha=0.05$). Homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 5.3. Mean leaf stomatal conductance (g_s) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliated treatments at Pittwater from late winter 2006 to autumn 2007. Error bars show mean standard errors ($\alpha=0.05$). Homogeneous groupings are identified by letters ($\alpha=0.05$).

Figure 5.4. Log-log transformed relationship between light-saturated photosynthetic rate (A_{\max}) and stomatal conductance (g_s) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliation treatments in an experimental plantation in Southern Tasmania. Trendlines are represented for pooled data in spring 2006 for undefoliated (solid line) and defoliated (dotted line) *E. globulus* trees.

Figure 6.1. Scatter diagrams of the measurements of canopy conductance (G_C) in the undefoliated treatment plotted against (a) air temperature (T), (b) air vapour pressure deficit (D) and (c) solar radiation (R), with the hypothetical boundary line fitted for each environmental variable.

Figure 6.2. Predicted versus observed canopy conductance (G_C) of *E. globulus* in the undefoliated treatment. The line is 1:1.

Figure 6.3. Daily variation in (a) rainfall, (b) air temperature (T) and (c) daily vapour pressure deficit (D) in *E. globulus* plantation in Southern Tasmania over the growing season of 2006/2007.

Figure 6.4. Typical profiles of soil matric potential in the experimental plot. Measurements were made on seven occasions. Irrigation commenced in early October 2006.

Figure 6.5. Mean predawn and midday leaf water potential (Ψ) of undefoliated and defoliated *E. globulus*. Data are presented for four measurement days: Oct 06, Dec 06, Jan 07, and Mar 07. Error bars show standard error ($\alpha=0.05$).

Figure 6.6. The relationship between maximum rates of transpiration (E_{\max}) with midday leaf water potential (Ψ_{md}) for undefoliated and defoliated *E. globulus* trees growing under similar atmospheric conditions.

Figure 6.7. Diurnal courses of transpiration (E , a and b), canopy conductance (G_C , c and d) in control and defoliated *E. globulus* trees on two contrasting days: 02/09/2006 (a,c,e), and 24/10/2006 (b,d,f) with respect to vapour pressure deficit (D , e and f). Data represent the mean and standard error of three trees ($\alpha=0.05$). For clarity, error bars are presented only for control trees.

Figure 6.8. Mean soil-to-leaf hydraulic conductance (K_P) of control and defoliated *E. globulus* trees in southern Tasmania. Data are presented for four occasions. Error bars show standard error ($\alpha=0.05$).

Figure 6.9. Scatter diagrams of the measurements of canopy conductance (G_C) in the control and defoliated treatments plotted against (a) air temperature (T), (b) air vapour pressure deficit (D) and (c) solar radiation (R), with the hypothetical boundary line fitted for each environmental variable.

Figure 6.10. Predicted versus observed canopy conductance (G_C) of *E. globulus* in the control and defoliated treatments. The line is 1:1

Figure 7.1. Means of (a) maximum photosynthesis (A_{\max}), and (b) leaf conductance (g_s) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees growing in Southern Tasmania. Errors bars show least square differences ($\alpha=0.05$).

Figure 7.2. Diurnal patterns of leaf water potential (Ψ) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees in (a) 03/01/2008, (b) 24/01/2008 and (c) 18/02/2008. Data represent the mean and standard error of measurement on three trees in each treatment during summer 2007/2008. To clarify the error bars are only represent at the bottom ($\alpha=0.05$).

Figure 7.3. Daily variation of (a) maximum transpiration rates (E_{\max}) and (b) maximum canopy conductance ($G_{C\max}$) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* at the Pittwater research plantation in southeast Tasmania over the growing season of 2007/2008. Arrows represent the time of the defoliation occurred.

Figure 7.4. A significant linear correlation between mean soil-to-leaf hydraulic conductance and (a) canopy averaged maximum photosynthesis (A_{\max}), and (b) mean A_{\max} from the upper crown in a sample of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus*. Values are presented for all measuring dates. Pooled data are presented by the solid line.

Figure 7.5. Mean increments of (a) height and (b) over-bark diameter for irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees. Errors bars show standard error differences ($\alpha=0.05$).

Figure 7.6. Measured versus predicted using CABALA (a) diameter at breast height (b) and (b) stem volume of rain-fed undefoliated, rain-fed defoliated, irrigated undefoliated, and irrigated defoliated *E. globulus* grown in trial plantation in Southern Tasmania. Seedlings were considered to be 0.1 m tall with 0.015 m² of leaf area. Solid trendline represents the linear regression for all data put together ($P<0.001$). On each plot the dotted line represents the 1:1 line.

Figure 7.7. Predicted entire stem volume of *E. globulus* growing in southern Australia, from planting to harvest at age 10 years. Values are presented for rain-fed undefoliated, rain-fed defoliated, irrigated undefoliated, and irrigated defoliated. Defoliation occurred each year at the same time period and at the same level.

Figure 8.1. Results of a power analysis to detect difference in growth between undefoliated and defoliated, based on a one-way ANOVA analysis, and $\alpha = 0.05$ (Lenth, 2009).

List of tables

Table 1.1. Summary of levels of defoliation found to significantly decrease growth (height diameter, and/or volume) of several evergreen tree species.

Table 1.2. Examples of leaf-chewing, skeletoniser and roller insects attacking *E. globulus* (Source: CRC pest database, 2009)

Table 1.3. Example of phloem sucker insect attacking *E. globulus* (Source: CRC pest database, 2009)

Table 3.1. Summary of significant ANOVA results showing degrees of freedom (*d.f.*), *F* and probability (*P*) for each analysis.

Table 3.2. Mean foliar non-structural carbohydrates contents for control and defoliated seedlings across zones 2 and 3 (Z2, Z3) 27 days after the defoliation. LSD homogeneous groupings are identified by letters ($\alpha=0.05$). Values were taken from Genstat.

Table 3.3. Mean values (\pm SE) of above-ground (stem, branch, leaf) mass ratio across each crown zone and for the three crown zones pooled together for control and defoliated seedlings 27 days after the defoliation. LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Table 4.1. Effects of defoliation on whole-tree mean stem growth, leaf area and branch variables of undefoliated and defoliated *E. globulus* trees 322 days after treatment imposed. Values are means of three replicates with SE in parentheses. ^ indicates whole-tree stem volume.

Table 4.2. Variation of mean above-ground biomass (stem, branch, foliage and branch plus stem, also named wood), foliage to branch dry mass (FWR) and biomass allocation in the lower (Z_L), middle (Z_M), and upper (Z_U) crown zones of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation 322 days after treatment. Number in parentheses are least squares standard errors. Treatments having different letters indicate significant differences within crown zones or root class at $\alpha=0.05$ using LSD test.

Table 4.3. Mean soluble sugars, starch and total non-structural carbohydrates concentrations (g mg^{-1}) of each organ in the lower (Z_L), middle (Z_M), and upper (Z_U) crown zones of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation in southern Tasmania measured at the harvest time (mid-winter). Treatments having different letters indicate significant differences within crown zones or root class at $\alpha=0.05$ using LSD test.

Table 5.1. Repeated measure ANOVA for effects of defoliation treatments on whole tree physiological response. Values are means of three replicates with SE in parentheses.

Table 5.2. Mean specific leaf area (SLA) of undefoliated and defoliated trees in lower inner, lower outer and upper crown zones from spring (Oct 06) to autumn (Mar 07). Standard errors are in parentheses.

Table 6.1. Summary of repeated-measured ANOVA on day basis showing degrees of freedom (*Df*), *F* and probability (*P*) and standard error (*SED*) for each analysis.

Table 7.1. Detailed CABALA site inputs for Pittwater Experimental research Station, southern Tasmania.

Table 7.2. Mean \pm SE plant hydraulic conductance (K_p) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees in plots growing under similar atmospheric conditions. Measurements were made on three occasions after the defoliation.

Definition of symbols

Symbols are listed under the chapter in which they are first used.

Chapter 1		
<i>Symbol</i>	<i>Description</i>	<i>Units</i>
G_C	Canopy conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
K_P	Soil-to-leaf hydraulic conductance	$\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$
A_S/A_L	Sapwood-to-leaf area ratio	
D	Vapour pressure deficit	kPa
$\Delta\Psi_{\text{pd-md}}$	Leaf water potential gradient between the soil and the leaf	MPa
η	Viscosity of water at the appropriate temperature	
h	height of the tree	m
Chapter 2		
$T_{\text{max}}, T_{\text{min}}$	Daily maximum and minimum temperatures	°C
T_a	Air temperature	°C
a.g.l	Above ground level	
R_H	Relative humidity	
V_S	Saturated water vapour pressure	kPa
NMM	Neutron moisture meter	
RCW	Relative water content	g g^{-1} ; MPa
P_a	atmospheric pressure	kPa
Chapter 3		
h	Height	m
d	Over-bark basal diameter	mm
A_{max}	Light-saturated photosynthetic rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_s	Stomatal conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
AEST	Australian Eastern Standard Time	h
Chl	Chlorophyll concentration	mg m^{-2}
N	Nitrogen content	g m^{-2}
P	Phosphorus content	g m^{-2}
SLA	Specific leaf area	$\text{m}^2 \text{kg}^{-1}$
h_{inc}	Height increment	m
d_{inc}	Over-bark basal stem diameter increment	mm
WUE	Instantaneous water-use efficiency	$\mu\text{mol mol}^{-1}$
NUE	Nitrogen-use efficiency	mmol mol^{-1}

$A:\text{Chl}$ A_{max} :chlorophyll concentration ratio $\text{mmol g}^{-1} \text{s}^{-1}$

Chapter 4

h	Height	m
d	Diameter at breast height	mm
SLA	Specific leaf area	$\text{m}^2 \text{kg}^{-1}$
A_L	Leaf area	m^2
$A_{\text{S0 1}}$	Under-bark cross-sectional area of sapwood at 0.1 m	m^2
$A_{\text{S1 3}}$	Under-bark cross-sectional area of sapwood at 1.3 m	m^2
ρ	Stem wood density	kg m^{-3}
ρ_{322}	Stem wood density 322 days later	kg m^{-3}
W_{CR}	Total coarse root biomass	kg
h_{inc}	Height increment	m
d_{inc}	Over-bark basal stem diameter increment	mm
V	Stem volume	m^3
h_z	crown zone length	m
A_{SZi}	sapwood area at the base of each crown zone	m^2
L_{Br}	Branch length	m
d_{ub}	Under-bark branch diameter	mm
d_{Br}	Over-bark branch diameter	mm
W_{Br}	Branch dry weight	kg
W_{F}	Foliage dry weight	kg
CSA	Branch cross-sectional area	m^2
W_{S}	Stem biomass	
W_{W}	Above-ground woody biomass	kg
W_{above}	Above-ground biomass	kg
W_{TREE}	Whole-tree biomass	kg
$A_L:CSA$	Ratio of leaf area per branch to branch CSA	kg
FWR	Foliage:branch dry mass	$\text{m}^2 \text{cm}^{-2}$
SSR	Shoot: root ratio	

Chapter 5

A_{max}	Light-saturated photosynthetic rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_s	Stomatal conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
AEST	Australian Eastern Standard Time	h
SLA	Specific leaf area	$\text{m}^2 \text{kg}^{-1}$

N	Nitrogen content	g m^{-2}
P	Phosphorus content	g m^{-2}
NUE	Nitrogen-use efficiency	mmol mol^{-1}

Chapter 6

J_s	sap velocity	$\text{g m}^{-2} \text{s}^{-1}$
Q	Water use was expressed on a tree basis	$\text{mmol m}^{-2} \text{hr}^{-1}$
E_L	Water use was expressed on per unit leaf area basis	$\text{mmol m}^{-2} \text{hr}^{-1}$
E	Transpiration per unit leaf area	$\text{mmol s}^{-1} \text{m}^{-2}$
Ψ_l	Leaf water potential	MPa
Ψ_{pd}	Pre-dawn leaf water potential	MPa
Ψ_{md}	Midday leaf water potential	MPa
E_{\max}	Maximum transpiration per unit leaf area	$\text{mmol m}^{-2} \text{s}^{-1}$
$G_{C\max}^j$	Maximum canopy conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
$K_G (T_A)$	conductance coefficient	$\text{kPa m}^3 \text{kg}^{-1}$
D	Vapour pressure deficit	kPa
K_p	Soil-to-leaf hydraulic conductance	$\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$
T	Air temperature	$^{\circ}\text{C}$
R	Daily total solar radiation	W m^{-2}

Chapter 7

h	Height	m
d	Diameter over-bark at 0.15 m above ground	mm
A_{\max}	Light-saturated photosynthetic rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_s	Stomatal conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
Ψ_{pd}	Pre-dawn leaf water potential	MPa
Ψ_{md}	Midday leaf water potential	MPa
Q	Water use was expressed on a tree basis	$\text{mmol m}^{-2} \text{hr}^{-1}$
G_C	Canopy conductance	$\text{mmol m}^{-2} \text{s}^{-1}$
E	Transpiration per unit leaf area	$\text{mmol s}^{-1} \text{m}^{-2}$
K_p	Soil-to-leaf hydraulic conductance	$\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$

Chapter 1. General Introduction.

1. 1. DEFOLIATING INSECTS: A CHALLENGE FOR CURRENT AND FUTURE FOREST GROWTH

Increased climatic variability, with extremely wet or extremely dry periods, may compromise health conditions and vulnerability to insect attack of forest ecosystems (Bréda and Badeau 2008). The potential impacts of defoliating pests on plantations health and productivity are expected to expand depending on the effects of climate change on the population dynamics of the defoliating pest, as well as the resultant condition and vigour of the trees (Old and Stone 2005). However, current knowledge on the increased risk of damage to forests and plantations by defoliating pest arising from changing climate is continually developing (Bale et al. 2002). Increased concerns exist that pests currently restricted to the warm tropical regions may move into temperate zone (Bale et al. 2002). For instance, in UK, some non-indigenous pest, such as gypsy moth (*Lymantria dispar* L.) and Asian longhorn beetle (*Anoplophora glabripennis*), are now found in southern Britain and may become a potential threat to UK forestry industry (Broadmeadow 2000). Warmer winters are also affecting the populations of defoliating by enhancing adult survival over-winter (Straw 1995). Climatic variability due to changing climate could increase the impact of defoliating pest attack, thereby compromising the health, and hence the carbon stocks, of Australian forests (Old and Stone 2005). In North America, climate change has been reported to contribute to the extent and severe outbreaks by the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), which may have considerable consequences on the ability of northern forests to assimilate and store atmospheric carbon (Kurz et al. 2008). Overall, an increase in extreme weather events is likely to exacerbate the impact of insect pests on plantations.

Among the challenges to be forest health faced by many countries, outbreaks of defoliating insects can be a major cause of natural disturbance, leading to decline in

forest ecosystems and resulting in the loss of economic and environmental resources. Decline conditions of trees have been associated with defoliation (Kulman 1971; Davidson et al. 1999), including eucalypt species (Wardell-Johnson et al. 2005). The most common symptoms of decline condition following defoliation are growth loss, production of smaller and fewer leaves, rotation delays, increased susceptibility to secondary insects, disease branches and tree mortality. Considerable literature exists on the detrimental effects of defoliation on growth and biomass production of evergreen trees (see Table 1.1). For example, defoliation of Douglas-fir (*Pseudotsuga menziesii*) reduced the overall radial growth considerably, the effect was carried beyond the year of defoliation (Brubaker and Greene 1979). Losses to mortality have been attributed to frequency and intensity of defoliation (Alfaro et al. 1982; Landsberg and Cork 1997).

1.2. GROWTH RESPONSE TO DEFOLIATION: WHAT DO WE KNOW?

1.2.1 Types of growth response

For categorising distinctly different patterns of response to silvicultural treatment, Snowdon (2002) established the concept of Type 1 and Type 2 responses. The time course of yield increment in hypothetical stands that exhibit Type 1 and Type 2 responses is illustrated in Figure 1.1.

The first is a Type 1 response where an initial decrease in growth is observed after tree damage, but is not sustained in the long-term and once the growth rate ceases to be directly affected by defoliation; defoliated and undefoliated stands follow parallel growth trajectories with a constant separation in time (Snowdon 2002). The second is a Type 2 response in which growth trends are indefinitely reduced resulting in a sustained change in tree productivity causing divergent growth trends from undefoliated trees (Snowdon 2002). A type 1 growth response is the ‘best case’ scenario whereas a type 2 is the ‘worst case’ scenario.

Table 1.1. Summary of levels of defoliation found to significantly decrease growth (height diameter, and/or volume) of several evergreen tree species. C refers to leaf-chewing insects and S to sap-feeding insects.

Tree species	Insect name	Type of insect	Level of leaf area removed (%)	Age at defoliation (years)	Tree height (m)	Authors
<i>Pseudotsuga menziesii</i>	<i>Choristoneura occidentalis</i>	C	57	3	1	(Chen et al. 2001; 2002)
<i>Pseudotsuga menziesii</i>	<i>Choristoneura occidentalis</i>	C	40	81	17	(Alfaro et al. 1982)
<i>Pseudotsuga menziesii</i>	<i>Choristoneura occidentalis</i> ; <i>Orgia pseudotsugata</i>	C	50	75 to 100		(Brubaker and Greene 1979)
<i>Pseudotsuga menziesii</i>	<i>Choristoneura occidentalis</i>	C	30	1		(Kolb et al. 1999)
<i>Pinus sylvestris</i>	<i>Diprion pini</i> ; <i>Neodiprion sertifer</i>	C	10	67.3		(Lyytikäinen-Saarenmaa and Tomppo 2002)
<i>Pinus sylvestris</i>	<i>Diprion pini</i>	C	20	70		(De Somviele et al. 2004)
<i>Pinus sylvestris</i>	<i>Diprion pini</i> ; <i>Neodiprion sertifer</i>	C	75	14.7	2.58	(Lyytikäinen-Saarenmaa 1999)
<i>Pinus contorta</i>	<i>Neodiprion sertifer</i>	C	46	6		(Britton 1988)
<i>Pinus ponderosa</i>	<i>Neodiprion gillettei</i>	C	60	1		(Sanchez-Martinez and Wagner 1994; 1999)
<i>Picea glauca</i>	<i>Zeiraphera canadensis</i>	C	30-60	10		(Carroll et al. 1993)
<i>Abies balsamea</i>	<i>Neodiprion abietis</i>	C	35	10 to 15	2 to 3	(Parsons et al. 2003)
<i>Abies balsamea</i>	<i>Choristoneura fumiferana</i>	C	90	30		(Piene 1980)
<i>Abies balsamea</i>	<i>Choristoneura fumiferana</i>	C	60	25 to 30		(Piene and Percy 1984)
<i>Abies grandis</i>	<i>Choristoneura occidentalis</i> ; <i>Orgia pseudotsugata</i>	C	50	75 to 100		(Brubaker and Greene 1979)
<i>Tsuga canadensis</i>	<i>Adelges tsugae</i>	C			6	(Stadler et al. 2005)
<i>Picea sitchensis</i>	<i>Elatobium abietinum</i>	S	40	1	0.35	(Straw et al. 2000; 2002)
<i>Picea sitchensis</i>	<i>Elatobium abietinum</i>	S	40	2	0.8	(Straw et al. 2005)
<i>Picea sitchensis</i>	<i>Elatobium abietinum</i>	S	20	10 to 18		(Day and McClean 1991)
<i>Picea sitchensis</i>	<i>Elatobium abietinum</i>	S	40		11 to 3.5	(Halldórsson et al. 2003)
<i>Eucalyptus globulus</i>	See Table 1.2	C	85	<1	0.34	(Floyd et al. 2002)
<i>Eucalyptus globulus</i>	<i>Perga affinis</i>	C	43	6		(Jordan et al. 2002)
<i>Eucalyptus globulus</i>	<i>Goniapterus scutellatus</i>	C	50	3	4.99	(Pinkard et al. 2006b)
<i>Alphitonia whitei</i>	<i>Casbia rectaria</i> ; <i>C. calliorma</i>	C	50	<1	0.28	(Jackson and Bach 1999)
<i>Eucalyptus blakelyi</i>	<i>Eriococcus coriaceus</i>	S	40-45	<1	0.42	(Vranjic and Gullan 1990)

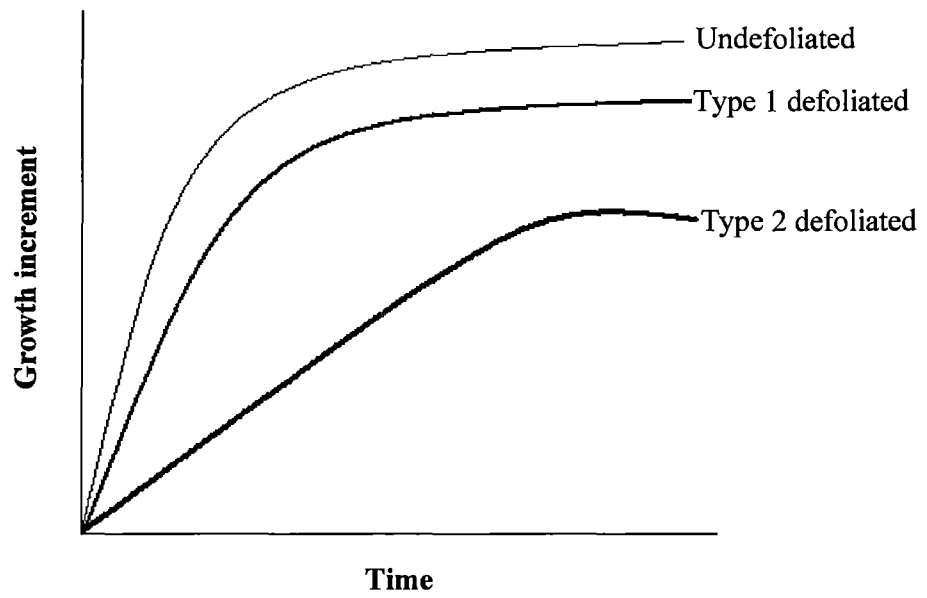


Figure 1.1. Type 1 and Type 2 responses to defoliation treatments show distinctive patterns in yield increment.

1.2.2 Variation in the growth response

Growth response of Douglas-fir in response to western spruce budworm has been studied extensively across a range of experimental conditions (Brubaker and Greene 1979; Alfaro et al. 1982; Kolb et al. 1999; Chen et al. 2001; 2002). The growth response of trees to foliage damage is variable. For example, in controlled conditions, Chen et al. (2002) reported that single 50%-defoliation of young seedlings had no significant impact on height increment and its relative growth; however it decreased basal stem diameter by 5% and the relative growth rate of basal stem diameter by 29%. The variability of the response depends on the effects of several interacting factors. One possible explanation for the difference between height and diameter growth response is that some buds partially consumed by the insects could still elongate. However, the information on the pattern of defoliation is not always available in literature, creating gaps in the interpretation of results. The level of defoliation may also explain the variability in the growth response. Kolb et al. (1999) showed that 60%-defoliation of the current-year foliage affected had a greater negative impact on growth response than 30%-defoliation of the current-year foliage.

In a comparison between two tree species in the same field site and with a similar level of defoliation by western budworm, Brubaker and Greene (1979) reported Grand-fir suffered more severely growth loss than Douglas-fir, which may suggest a genetic variation in tree resistance to insects, or differences in the interaction between plant and insect. Chen et al. (2001) demonstrated that effect of budworm on growth response varied among trees of the same species. They found that phenotypic differences in crown condition of Douglas-fir following budworm defoliation were influenced by tree genotype and that high growth rate and late bud burst phenology promoted tree resistance to budworm defoliation. Growth responses also varied with soil water and nutrient availability. In controlled conditions, Joly et al. (1989) showed that growth response of Douglas-fir seedlings depending on soil moisture, reporting greater proportional growth of roots versus shoots for water-stressed seedlings compared with non-stressed seedlings.

How insects influence plant growth response may also depend on the type of injury (Welter 1989). Traditionally, insects have been placed in feeding guilds according to their taxonomic status. The two main feeding guilds of defoliating pests have been identified as leaf-chewer and sap-feeder insects. Leaf-chewers are herbivores that remove partially or entirely leaf by action of the mandibles. They mainly include: beetle (Coleoptera), budworm and moth (Lepidoptera), sawfly (Hymenoptera). Sap-feeders, including aphids, scale insects, psyllids (Hemiptera) and mites (Arachnida) feed on sap drawn directly from the leaf's vascular system. Sap-feeders insects reduce leaf function without reducing area. Different kinds of insects could have different effects on growth; yet comparative studies of plant responses to different types of damage are rare. In Douglas-fir, Smith and Schowalter (2001) found that feeding by sap-feeders affected root growth to a greater extent than shoot growth. In contrast, defoliation by leaf-chewer budworm affected stem growth, but not the roots (Kolb et al. 1999). The siphoning of large amounts of carbohydrates from plants by sap-feeders showed to affect long-term growth and there was no sign of recovery (Smith and Schowalter 2001). Removal of leaf area appeared to be more readily tolerate with sign of recovery within few years following the loss of growth (Brubaker and Greene 1979).

The timing of defoliation may be important in determining the growth response to defoliation. In a study using two sawfly species, *Diprion pini* (Linnaeus) and *Neodiprion sertifer* (Geoffroy) that attack on Scots pine at different time in the season, early summer defoliation by *N. sertifer* suppressed growth more than late summer defoliation by *D. pini* (Lyytikainen-Saarenmaa 1999; Lyytikainen-Saarenmaa and Tomppo 2002). In contrast, in artificially-defoliated Scots pine, early defoliation reduced stem growth less than late defoliation (Ericsson et al. 1980).

1.2.3. Physiological approach: can it explain the growth response?

As shown by the two previous examples, it appears impossible to generalise a single growth response to foliage loss. In response to defoliation, plants are able to induce resistance mechanisms which consist in the activation of the host plant's own

genetically programmed defence pathways, resulting in changes that diminish the effects of subsequent biotic attack (Agrawal 1998; Eyles et al. 2009a). Plants are able to minimize the negative fitness consequences of tissue lost to herbivory by activating physiological processes that allow the plant to compensate for the reduction in total photosynthetic capacity. These are known as inducible ‘civilian’ defences (Eyles et al. 2009a). Understanding general patterns to these responses, and linking these patterns to underlying physiological mechanisms, could provide a common basis to determine how trees respond to insect-induced stress (Welter 1989; Peterson and Higley 1993). Insect feeding can directly or indirectly influences gas exchange processes, including photosynthesis, stomatal conductance and transpiration, and foliar traits and chemistry (Figure 1.2).

Leaves are the source of carbon for the plant. By removing leaves through defoliation, the balance between carbon source and carbon sink (active zones of growth) is altered, and this is commonly used to explain why photosynthetic rates often increase in remaining leaves in response to defoliation. Therefore, defoliation is expected to affect source and sink dynamics. An up-regulation of photosynthesis in response to defoliation is not a universal response. While it is commonly observed following insect defoliation, defoliation associated with sap-feeder insects may in fact result in a down-regulation of photosynthesis. For example, constant feeding pressure by scale insects resulted in lower leaf gas exchange of hammock tree (*Guaiacum sanctum* L.) presumably due to the decreased stomatal conductance (Schaffer and Mason 1990). Yet, the mechanisms to explain the photosynthetic responses are not clearly defined. In some studies using artificial manipulation on *E. globulus* it has been concluded that that increased photosynthesis following defoliation was a function of changes in the biochemical processes of photosynthesis (Pinkard and Beadle 1998c; Turnbull et al. 2007)). In Douglas-fir, increased photosynthesis was related to increased stomatal conductance as a result of a more favourable balance between leaf and root area (Chen et al. 2001), or related to increase in foliar nitrogen concentration (Kolb et al. 1999). Few studies have also shown an improvement in plant water status *via* the enhancement of leaf water potential following defoliation (Kolb et al. 1999), or the increase in plant hydraulic conductance (Reich et al. 1993).

The way in which biomass is partitioned between different plant parts reflects the relative strength of areas of carbon utilisation within the plant (Cannell 1985), as well as a functional balance between different plant parts. Following defoliation, trees favoured increased allocation to shoot growth (Kolb et al. 1999; Pratt et al. 2006), this until the root/shoot ratio prior to defoliation is again achieved (Pratt et al. 2006). The release from apical dominance, primarily from the removal of buds, has been shown to increase plant growth response (Chen et al. 2002; Pratt et al. 2006), therefore play an important role in the compensatory growth (Belsky 1986).

Death of *Eucalyptus* species after repeated defoliation by *Glacaspis* spp. was related to the exhaustion of carbon reserves to a level which did not support respiration and growth (Bamber and Humphres 1965). The removal of photosynthetic tissue by insect defoliation has been documented to lower carbohydrate storage reserves (e.g. Bamber and Humphres 1965; Webb 1981; Piene and Percy 1984; Hudgeons et al. 2007). According to the source-sink hypothesis, the primary way that defoliation affects the plant is to change the ability of the meristems to compete for resources (Honkanen et al. 1994). To recover from defoliation, trees need adequate carbohydrate reserves to support activity of new sinks and regenerate new leaf tissue (Webb 1981; Hudgeons et al. 2007). Responding to herbivores, significantly higher quantities of carbon-based leaf chemical defences, such as tannins and other phenolics, were found in leaves of defoliated Scots pine (Lyytikainen-Saarenmaa 1994). The production of phenols is usually considered to be potent anti-herbivore chemicals because they form indigestible complexes with proteins and have the potential to reduce the nutritive value of plant food. These mechanisms are also known as inducible chemical defences (Eyles et al. 2009a).

In addition, by the removal of transpiring leaf surface, insect herbivores are expected to affect plant water use. Yet, very little is known about the impact of insect herbivores on tree water status (Salleo et al. 2003) and water use (Cunningham et al. 2009). Salleo et al. (2003) reported that horse-chestnut tree (*Aesculus hippocastanum*) responded to leaf miner *Cameraria ohridella* defoliation by improving the hydraulic efficiency of the conducting tissue, thus ameliorating the water and nutrient supply per unit leaf area and, therefore, partially compensating for the reduction of leaf area. In *Eucalyptus*

blakelyi, Cunningham et al. (2009) argued that the effect insect herbivore would have a detrimental impact on tree performance, leading to substantial increase in water use. Controversially, studies have also reported increased in water-use efficiency following defoliation (Ellsworth et al. 1994).

1.2.4. Role of site factors in responses to defoliation

Tree responses to defoliation are extremely complex (see Figure 1.2) and are likely to be influenced by external factors such as site conditions (see Figure 1.3). For example, herbivory studies have demonstrated that physiological compensatory response occurs when nitrogen is supplied to defoliated plants, or soil moisture is adequate (Prins and Verkaar 1992). However, the present insight of growth response to defoliation is unclear because studies failed to address interactions between effects of defoliation and abiotic stressors on trees. Only a few studies have shown that defoliation, natural and artificial, can result in increased water availability and alleviate impact of drying soil on growth (Stephens et al. 1972; Welker and Menke 1990; Kolb et al. 1999), although the response was not consistent with other studies (e.g. Osman and Sharrow 1993). Up-regulation of photosynthesis may also depend on water (McGraw et al. 1990), or soil nutrient availability (Lovett and Tobiessen 1993; Pinkard et al. 2007). For example, the additional supply of N increased carbohydrate production by increasing photosynthesis, and these carbohydrates were used to rebuild tree crowns, thereby increasing the potential productivity of the crowns (Pinkard et al. 2007). However, studies also showed that up-regulation of photosynthesis were not related to supply in nitrogen (Ovaska et al. 1993b).

It has been commonly assumed that plants are generally best able to recover from herbivory when growing in high resource conditions, an assumption which is supported by some models (e.g., the continuum of responses model, CRM) but opposed by others (e.g., the growth rate model, GRM). The CRM (Maschinski and Whitham 1989) predicts that the compensation for herbivore increases with increasing nutrient levels. At high nutrient levels, tissues can be replaced more readily and plants can grow faster so as to recover more rapidly from damage. In contrast, the GRM

(Hilbert et al. 1981) predicts that when plants, growing below their potential maximum relative growth rate at low resource levels, require only small changes in growth to compensate for herbivore. Hawkes and Sullivan (2001) showed in a growth meta-analysis of 45 studies including 17 studies on woody plants that recovery from significant herbivore damage was more rapid in low resource conditions. Collectively, it appears difficult to ascertain that growth of trees growing in low productive sites would be more affected by the defoliation. Part of the explanation may be beyond the host-insect interaction continuum.

1.3. THE PROJECT

1.3.1. *Eucalyptus globulus* plantations

Worldwide, eucalypt plantations exceed 20 million hectares and this is expected to increase to meet growing demand for wood and other forest-based products (1997; Macfarlane et al. 2004; Fernandez et al. 2006). In Australia, total plantation area now exceeds 1.97 million hectares (Mha), and is constantly expanding. The hardwood plantation estate is 0.95 Mha (Bureau of Rural Sciences 2009), with 62% of this comprising *E. globulus*. More than 70% of these plantings are in Tasmania, Victoria and Western Australia. *Eucalyptus globulus* Labill., a fast growing tree that is native to south-eastern Tasmania, the Bass Strait Islands and south-eastern Victoria (Chippendale 1988), is primarily planted for pulpwood (Eldridge et al. 1993). The species has a number of highly desirable qualities including high growth rates (Downes et al. 1999), relatively high drought tolerance (White et al. 1999) and good fibre qualities (Beadle et al. 2000) making it ideal for commercial production. The demand for increased timber production in Australia and for a new primary source of hardwood that provides high-quality pulpwood has resulted in the rapid expansion *E. globulus* plantations in the south of the country (Macfarlane et al. 2004; Bureau of Rural Sciences 2008).

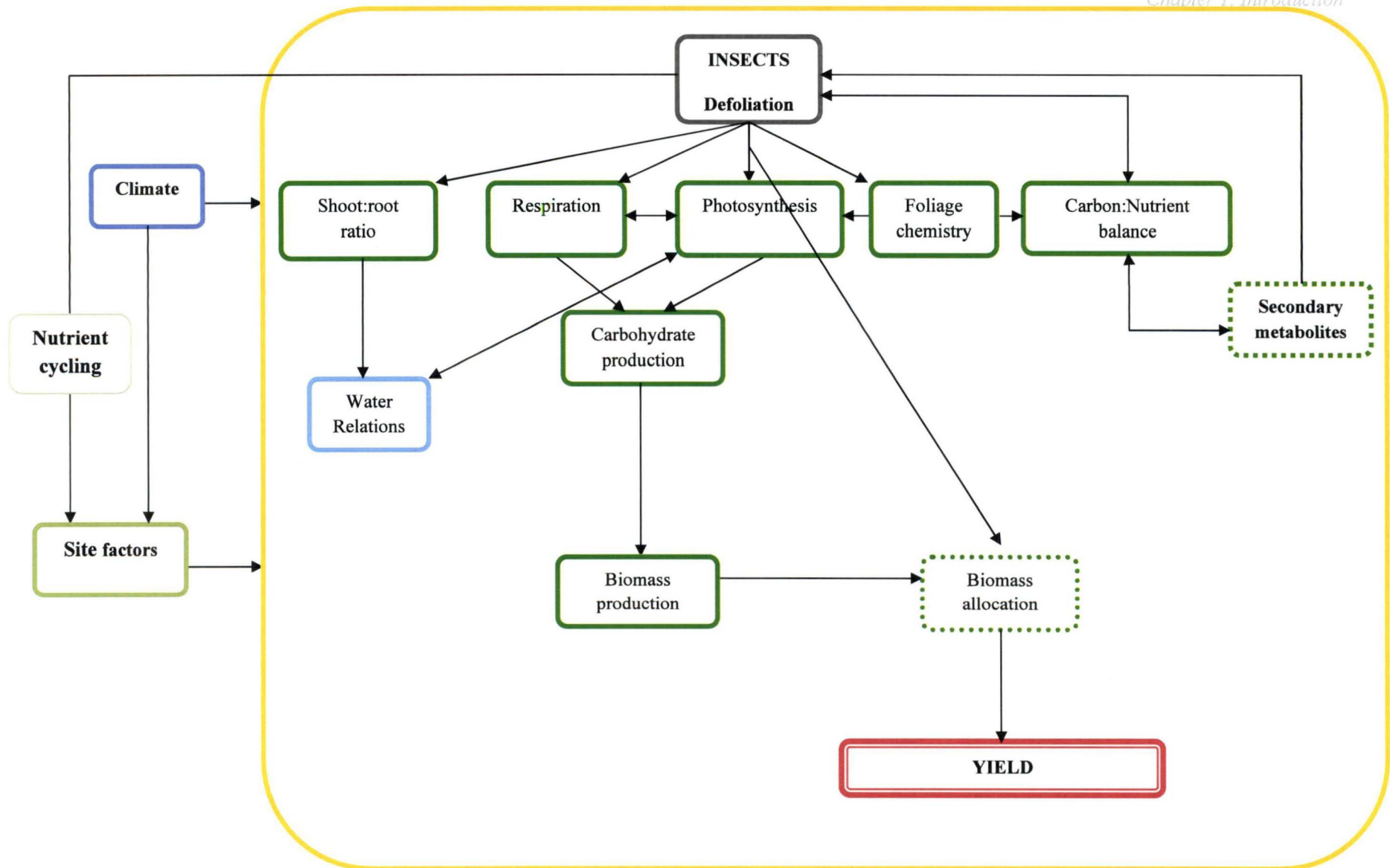


Figure 1.2. Effects of internal factors on the physiological response of plant to defoliation.

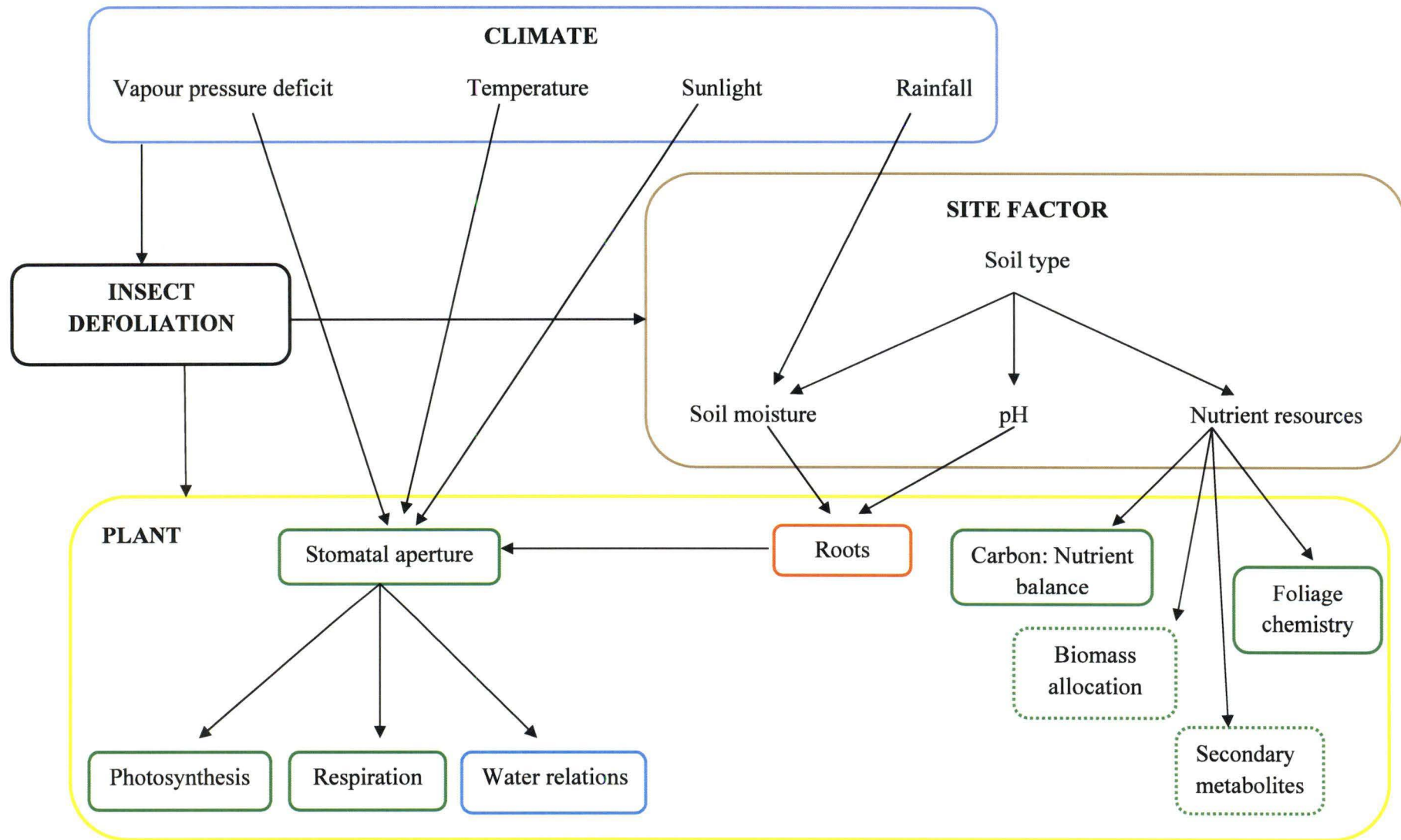


Figure 1.3. Effects of external factors on the physiological response of plant to defoliation.

1.3.2. Insect defoliation in *E. globulus*

To the rapid expansion *E. globulus* plantations, there has been a corresponding increase in serious insect outbreaks in *E. globulus* plantations (Ohmart and Edwards 1991; Abbott 1993; Bashford 1993; Neumann 1993; Stone 1993; Elek 1997; Elliott et al. 1998; Lanfranco and Dungey 2001; Loch and Floyd 2001; Neumann et al. 2005; Fernandez et al. 2006). Defoliation represents a major risk to forests productivity as it may lead to a reduction of the potential volume increment per unit time and is of major concern to an industry in which maximising production over short rotations is the key to market competitiveness (Candy et al. 1992; Elliott et al. 1993).

The most important defoliators of *E. globulus* plantations are listed in Tables 1.2 and 1.3. Leaf-chewing insects are among the most destructive pest in *E. globulus* plantations, but there are also significant attacks by sap-feeders. Defoliation occurs most often in spring and summer, and sometimes in autumn (CRC 2009). These herbivores feed on the leaves, new growth and buds of *E. globulus*, some showing preference for either juvenile and/or adult foliage (CRC 2009). Economic losses through reductions in tree height, diameter and volume, and potential growth malformation can arise from such damage, as demonstrated with *P. bimarum* Olivier in Tasmania (Candy et al. 1992; Elek 1997). An understanding of physiological and morphological responses to defoliation will improve the capacity to model the impact of defoliation in determining stand productivity and wood quality in current and future climate.

1.3.3. Scope of the project

There is also a need to better understand the potential of interactive effects between abiotic stressors and defoliation on plant physiological response. With increasing likelihood of insect outbreaks and the associated changes in climate, particularly predicted increased drought intensity and frequency (IPCC 2001), there is an imperative to better define the effects of pest defoliation under a range of environmental conditions, particularly the impacts on regional carbon and water

balances (Battaglia et al. 2004). Studies of induced resistance to defoliation have commonly investigated physiological responses to loss of leaf area, but often have ignored the response from mature trees in canopy closure condition and the interaction with other environmental stress factors such as drought. This project was established with the intention of identifying the physiological mechanisms driving plant stress response to defoliation by leaf-chewing insects in different soil moisture conditions. The project involved a study of growth and physiological responses of young and mature *E. globulus* trees following artificial defoliation, and the examination of the implications of interactive effects between low water supply and defoliation on tree rotation-length growth and water use. While the project was confined to a trial plantation, investigation of physiological responses in both pot and field experiments ensured that the general principles developed would have broad applications. The results and conclusions are relevant to commercial and non-commercial forest management.

1.4. HYPOTHESES IN PLANT DEFOLIATION

For the purpose of this project, I intended to investigate four major hypotheses that are currently proposed to explain plant responses to insect defoliation.

Hypothesis 1. Artificial defoliation simulates insect defoliation

Often, the most important consequence of defoliation to plants is believed to be the loss of leaf tissue. Under this assumption, many experimental studies have relied on artificial defoliation to simulate insect herbivore defoliation. It has been clearly demonstrated in the paragraph above that plant responses to insect defoliation are complex and variable, and could sometimes be challenging logistically.

Table 1.2. Examples of leaf-chewing, skeletoniser and roller insects attacking *E. globulus* (Source: CRC pest database, 2009)

Scientific name	Order (family)	Period of damage	Susceptible Plant Parts	Spatial Pattern	Authors
<i>Anoplognathus chloropyrus</i> (Christmas Beetle)	Coleoptera (Scarabaeidae)	Summer	juvenile and adult leaves	patchy	Thomson et al. 2001
<i>Cadmus excrementarius</i> (Leaf Beetle)	Coleoptera (Chrysomelidae)	Summer	new growth , juvenile and adult leaves		Loch & Floyd 2001; Loch 2005 & 2006
<i>Gonipterus scutellatus</i> spp. (Eucalyptus Snout Weevil)	Coleoptera (Curculionidae)	Spring-Summer-Autumn	new growth , juvenile and adult leaves	uniform	Collett 2001; Loch & Floyd 2001; Loch 2005 & 2006
<i>Heteronyx elongatus</i> (Scarab Beetle)	Coleoptera (Scarabaeidae)	Spring-Summer-Autumn	new growth , adult leaves, root and seedling	patchy	Loch & Floyd 2001
<i>Liparetrus jenkinsi</i> (Leaf Beetle)	Coleoptera (Scarabaeidae)	Spring	juvenile and adult leaves, and seedling	patchy	Loch & Floyd 2001
<i>Mnesampela privata</i> (Autumn gum moth)	Lepidoptera (Geometridae)	Autumn	juvenile leaves	edges	Loch & Floyd 2001; Floyd et al. 2002
<i>Paropsisterna agricola</i> (Leaf Beetle)	Coleoptera (Chrysomelidae)	Spring-Summer	new growth , juvenile and adult leaves	patchy	Collett 2001; Loch & Floyd 2001; Nahrung et al. 2004; Loch 2004
<i>Paropsisterna bimaculata</i> (Leaf Beetle)	Coleoptera (Chrysomelidae)	Summer-Autumn	new growth , juvenile and adult leaves	patchy	Collett 2001; Loch & Floyd 2001; Nahrung et al. 2004; Loch 2005
<i>Paropsisterna nobilitata</i> (Leaf Beetle)	Coleoptera (Chrysomelidae)	Spring-Summer-Autumn	new growth , juvenile and adult leaves	patchy	Loch 2005
<i>Perga affinis</i> (Green Sawfly)	Hymenoptera (Pergidae)	Autumn-Winter-Spring	juvenile and adult leaves	patchy	Jordan et al. 2002
<i>Phaulacridium vittatum</i> (Wingless Grasshoper)	Orthoptera (Acrididae)	Spring-Summer	juvenile and adult leaves, and stem	patchy	Loch & Floyd 2001
<i>Uraba lugens</i> (Gum leaf Skeletoniser)	Lepidoptera (Noctuidae)	Spring-Summer	juvenile and adult leaves, and lower crown	patchy	Collett 2001
<i>Stiepsicrates macropetana</i> (Leaf rollers)	-		adult leaves		Collett 2001

Table 1.3. Example of phloem sucker insect attacking *E. globulus* (Source: CRC pest database, 2009)

Scientific name	Order (family)	Period of damage	Susceptible Plant Parts	Spatial Pattern	Author
<i>Amorbus</i> spp. (Coreid bug)	Hemiptera (Coreidae)	Spring-Summer-Autumn	new growth		Collett 2001
<i>Eriococcus</i> spp. (Gum tree scale)	Hemiptera (Coccoidea)	All year	juvenile and adult leaves, and stem	patchy	Collett 2001
<i>Ctenarytaina eucalypti</i> (Bluegum Psillid)	Hemiptera (Psyllidae)		new growth and juveniles leaves	patchy	Collett 2001
<i>Nysius vinitor</i> (Rutherglen Bug)	Hemiptera (Lygaeidae)	Early summer	juvenile and adult leaves	patchy	Loch & Floyd 2001

In contrast, artificial manipulations are assumed to have significant advantages (Baldwin 1990), including: (1) they are less time consuming, (2) severity of the damage is readily controlled, (3) there is a reduced possibility of inadvertently introducing pathogens, and (4) it is possible to assign the treatment across plant samples randomly. However, the adequacy of the artificial method has been questioned in terms of accuracy and differences in the responses may have been revealed (Baldwin 1990; Lyytikäinen-Saarenmaa 1999).

In this project, and like many previous studies, the main experiments investigating tree responses to defoliation utilised artificial defoliation rather than natural defoliation for logistical considerations. However, the following question arose:

- ✓ does artificial defoliation accurately simulate insect defoliation?

Therefore, the following hypothesis was tested:

- *E. globulus* responds similarly to both artificial and insect defoliations.

Hypothesis 2. Source-sink hypothesis

Physiological responses to defoliation are complex and dependent on the timing of the damage, fraction of crown affected, and the severity of the damage. Studies of induced-defoliation demonstrated clearly that defoliation modifies the dynamic between carbon sources and sinks, which leads to change in source:sink balance and ultimately to poor plant growth. Honkanen et al. (1994) proposed two main reasons to investigate the effect of defoliation:

- (1) Understanding the mechanisms driving the modification of source/sink dynamics in tree following defoliation may be important in interpreting, predicting and modelling impact on tree growth and production,

(2) Insect herbivores, such as leaf beetles, may remove leaves, which are sinks when young but later change to source when older, and the removal of sources or sinks from a tree may have different effects (Haukioja et al. 1990).

In this project, the effects of defoliation on source-sink hypothesis were tested to better understand the mechanism driving the physiological compensatory response to partial defoliation. The following questions were addressed:

- ✓ are physiological mechanisms adopted by *E. globulus* in response to a single defoliation event?
- ✓ do the strategies showed by *E. globulus* affect growth response and tree water use?

The hypotheses tested were:

- defoliation results in up-regulation of photosynthesis
- the physiological responses determine the impact of defoliation on growth.

Hypothesis 3. Resource limitation hypothesis

Although it is widely accepted that a plant's tolerance of herbivore damage depends on resource availability in the plant's environment, there is no consensus on whether higher resource levels lead to greater or to lower tolerance. In this project, the effects of resource supply on responses to partial defoliation were tested better tolerate defoliation in condition of high resources levels i.e. with supply of water *via* irrigation and nutrient *via* fertilisation, or in rain-fed condition. The following questions were addressed:

- ✓ does *E. globulus* better tolerate defoliation in conditions of high resource levels with adequate water and nutrient supply, or in condition of low water supply?
- ✓ does low water supply limitation affect *E. globulus* responses to defoliation?

The hypotheses tested were:

- the supply of water *via* irrigation supports *E. globulus* growth and compensation following defoliation.
- the rain-fed condition impairs *E. globulus* capacity for growth and compensatory responses to defoliation.

Hypothesis 4. Hydraulic model

Whole-plant hydraulic conductance has been described by the following hydraulic model (Whitehead and Jarvis 1981; Ewers et al. 2005):

$$G_C = K_P \cdot \frac{A_S}{A_L} \cdot \frac{1}{D} \cdot (\Delta\psi_{pd-md} - h\eta)$$

where G_C is mean canopy stomatal conductance, K_P is soil-to-leaf hydraulic conductance per unit sapwood area, A_S/A_L is sapwood-to-leaf area ratio, D is vapour pressure deficit, $\Delta\psi_{pd-md}$ is water potential gradient between the soil and the leaf, and $h\eta$ is the viscosity of water at the appropriate temperature and h the height of the tree. Partial defoliation is expected to increase A_S/A_L , for a given D , and in most case, transpiration rate per unit leaf and canopy conductance have generally been found to also increase (Reich et al. 1993; Pataki et al. 1998). The response has been attributed to the adjustment in K_P (Meinzer and Grantz 1990).

In this project, the impact of defoliation on tree water status was tested to validate the hydraulic model. By reducing leaf area, it is expected to also impact tree water use.

The following hypotheses were tested:

- low water supply limits tree recovery following defoliation
- defoliation ease the negative effect of low water stress on growth of *E. globulus*

Chapter 2. Description of the Pittwater experiments

This project was divided into three experiments. The first experiment was carried out in controlled glasshouse conditions in Newtown, Southern Tasmania (Chapter 3). Description of the pot experiment is presented in Chapter 3. The two main experiments were conducted in a *E. globulus* experimental plantation (Chapters 4, 5, 6 and 7) located 20 km east of Hobart, Australia (42° 49.4'S 147° 30.6'E). The description of the site is presented below. The plant material used in the first field experiment was used in the chapters 4, 5 and 6. The plant material used in the second field experiment was used in the chapter 7.

2.1. SITE DESCRIPTION

The site has a gentle slope of 2% running NW to SE. Climate at the site is cool temperate maritime, with an average rainfall of approximately 500 mm per year, annual evaporation is in excess of 1300 mm per year. Daily maximum and minimum temperatures (T_{\max} , T_{\min}) range between 22.5 and 12.5°C in summer and 12 and 4°C during winter (Australian Bureau of Meteorology, www.bom.gov.au).

The site had uniform fine sandy soil profile (1–2 m deep), bulk density throughout the profile approximately 1400 g mm⁻³ and trees had access to a number of potential water sources including town water for irrigation and fresh groundwater. Prior to establishment, the site was used as a pony arena for over 10 years during which there was no vegetation, as a result the soil profile was free from roots.

2.2. PLANT MATERIALS

2.2.1. Field experiment 1

In September 2002, *E. globulus* seedlings were planted in nine plots each containing 25 trees covering an area of 225 m². An electrified, rabbit-proof fence was erected to exclude large herbivores. Seedlings were sourced from the Gunns Woolnorth seedling orchard in North-west Tasmania and had been raised from seed in Lannen 81f trays containing 8.5×10^{-5} m³ of composted pine bark and peatmoss. Each seedling was planted in a hole prepared using a hand trowel, aimed at providing uniformity in planting conditions and watered immediately. Seedlings were planted by hand on 3×3 m spacing, equivalent to 111 stems ha⁻¹, using a three-way Latin square design consisting of three water availability treatments (O'Grady et al. 2005).

The drip irrigation system provided water to both sides of the seedlings at the rate of 6 mm every second day. Seedlings were fertilised two weeks after planting, and at 3-monthly intervals following planting. Granulated fertiliser was applied evenly across the site at 3 monthly intervals at an equivalent rate of 106 kg ha⁻¹ year⁻¹ nitrogen, 59 kg ha⁻¹ year⁻¹ phosphorus and 60 kg ha⁻¹ year⁻¹ potassium, also including a suite of trace elements. Weed control was maintained throughout growth to maintain a weed free site using a combination of manual weeding and chemical control. Glyphosate was applied where and when necessary to control the weeds sorrel and capeweed.

2.2.2. Field experiment 2

The second trial was planted on a 3 m by 3 m spacing in two experimental watering regimes and replicated using a completely randomised split-plot design (Eyles et al. 2009b). *E. globulus* seedlings of 0.25 m height were established in December 2006. During the two first months, all seedlings were irrigated every second day using municipal water at a rainfall equivalent of 3 mm. In February 2007, the rain-fed (control) treatment was applied in half the plots. Thus control plots received rainfall only. The irrigated plots received municipal water at a rainfall equivalent of 6 mm

every second day. All seedlings were fertilised two weeks after planting, and at three-monthly intervals following planting at an equivalent rate of 100 kg N ha⁻¹ year⁻¹ and 60 kg P ha⁻¹ year⁻¹ (O'Grady et al. 2005).

2.3. BIOPHYSICAL ENVIRONMENT

Climate variables were monitored using an automatic weather station installed in an open field approximately 100 m north-west of the plantation. Air temperature (T_a) and humidity were measured using a temperature/humidity probe (Vaisala HMP35A, Helsinki, Finland) mounted 1.5 m above ground level inside a Stevenson screen. Total radiation was measured at 2 m above ground level using a pyranometer (Licor LI200X, Lincoln, Nebraska, USA). Wind speed and direction were measured at 2 m above ground level using an anemometer Met One 014A (Met One Instruments Inc., Grants Pass, Oregon, USA); and rainfall was measured using a 200 mm tipping bucket (0.2 mm per tip) rain gauge (Monitor Sensors TBRG, Caboolture, Qld., Australia). Climate data were recorded every minute. Thirty-minute averages of all variables except rainfall were logged using a CR10 data logger (Campbell Scientific, Logan, Utah, USA). For rainfall, the 30 minute total was recorded. Vapour pressure deficit (D) was calculated from relative humidity (R_H) and air temperature following Goff and Gratch (1946) equations (Eq. 2.1; 2.2):

$$V_s = 0.611e^{(17.27T_a/T_a+237)} \quad (\text{Eq. 2.1})$$

$$D = \left(\left(\frac{R_H}{100} \right) V_s \right) - V_s \quad (\text{Eq. 2.2})$$

where V_s is saturated water vapour pressure. In addition to these climatic variables, atmospheric pressure (P_a) (for calculation of canopy conductance) was obtained from the Bureau of Meteorology and measured at Hobart Airport, less than 1 km away and at a similar elevation above sea level.

Soil moisture content was measured several occasions using a neutron moisture meter (Hydroprobe, CPN-503, Santa Barbara, CA, USA). An access tube was installed in the middle of the plot and neutron moisture meter counts were recorded at 150–200 mm intervals to approximately 2 m below ground level. Calibrations between relative water content (*RWC*) and neutron moisture meter counts developed for soils at the site were used to convert counts to relative water content (g g^{-1}). *RWC* was converted to matric potential (MPa) based on a soil water release curve developed for soil at the site (Worledge, D., O’Grady, A.P., unpublished data).

Chapter 3: Natural and Artificial Defoliation.

3.1. INTRODUCTION

Logistic constraints on conducting experiments related to insect defoliation in the field make assessments of herbivory on plants hosts difficult. An alternative option consists of simulating herbivory through mechanical defoliation and studying plant responses. Such an approach offers significant insights into the ecological, physiological and morphological responses of plants to herbivory and also provides convenient way to test plant responses to various types and levels of defoliation (Hjältén 2004; Lehtilä and Boalt 2004). Additionally, artificial defoliation trials are statistically reliable as the exact magnitude and distribution of defoliation can be controlled and measured (Hjältén 2004). In the context of developing tools for pest management artificial defoliation facilitates the acquisition of fundamental knowledge by providing an explicit a priori basis of plant responses (Raghu and Dhileepan 2005; Conrad and Dhileepan 2007).

However, it is generally impossible to mimic exactly the type of damage that may occur for insect defoliation. Artificial defoliation methods are often selected based on logistical considerations such as time taken to defoliate plants, and may bear no resemblance to the spatial and temporal patterns of insect damage (Heichel and Turner 1983; Ovaska et al. 1992; Ovaska et al. 1993b; Reich et al. 1993; Vanderklein and Reich 1999). Artificial defoliation studies with *E. globulus* have involved removal of entire leaves whereas this rarely occurs with its main defoliating pests (Pinkard et al. 2007; Turnbull et al. 2007).

Even though simulated herbivory may provide a good indication of the direction of a plant's response to a given type of damage, it is impossible to replicate real herbivore damage accurately and several problems have been identified (Baldwin 1990; Hjältén 2004; Lehtilä and Boalt 2004). For instance, artificial defoliation is often done at a

single point in time that fails to adequately simulate a normal larval feeding period (Ericsson et al. 1980). The cutting action of larval mouthparts potentially may act in a different manner to instruments used for artificial defoliation (Agrawal 1998). Larvae may transfer fungal or bacterial pathogens in addition to any direct effect of saliva (Dyers and Bokhari 1976). In addition, frass may contribute to nutrient recycling (Lightfoot and Whitford 1990). Baldwin (1990) has indicated a differential sensitivity to artificial and natural defoliation, even when the former is rigorously matched to the mechanism of natural defoliation. Therefore the following question arises: how different are plant responses to artificial defoliation from those induced by herbivory?

Previous studies have compared the two approaches in slow-growing evergreen conifer trees including lodgepole pine [*Pinus contorta* Dougl. ex Loud.] (Britton 1988), balsam-fir [*Abies balsamea* (L.) Mill.] (Piene and Little 1990), ponderosa pine [*Pinus ponderosa* Dougl. ex Laws.] (Sanchez-Martinez and Wagner 1994), Scots pine [*Pinus sylvestris* L.] (Lyytikainen-Saarenmaa 1999) and fast-growing evergreen conifer trees such as Douglas-Fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Chen et al. 2002). These studies have only examined responses in terms of impacts on growth and biomass allocation, and sought not sort to address the underlying processes driving these responses. Furthermore these findings have not always been consistent, with both similar (Britton 1988; Sanchez-Martinez and Wagner 1994; Chen et al. 2002) and contrasting (Baldwin 1990; Lyytikainen-Saarenmaa 1999) effects reported, suggesting for the need of deeper mechanistic understanding. For instance, increase in photosynthetic rate along with changes in foliar nitrogen and carbohydrate contents commonly occur following defoliation events in a large range of tree species (Hoogesteger and Karlsson 1992; Ovaska et al. 1993b; Reich et al. 1993; Pinkard et al. 1998; Vanderklein and Reich 1999; Chen et al. 2001).

The recent increased activity of *Paropsisterna agricola* in southern Australia has been facilitated by rapid expansion in plantings of *E. globulus* and *E. nitens* (Dean and Maiden). Currently, *P. agricola* costs annually the timber industry AU\$12 000 in pesticide applications, and up to AU\$870 per hectare in lost production through reductions in tree height, diameter and volume, and potential growth malformation (Nahrung 2003). Here I examined effects of *P. agricola* feeding on the juvenile sessile

leaves and buds of *E. globulus* seedlings. It is not known whether artificial defoliation can be used to simulate the effects of *P. agricola*. This experiment compared the effects of artificial and insect defoliation by larvae of *P. agricola* on mean CO₂ assimilation, stem growth and above-ground biomass production, and foliar chemistry in *E. globulus* seedlings. Two hypotheses were tested that:

1. seedlings respond to defoliation by changing physiological processes to compensate the loss of foliage and,
2. there are differences between the effects of artificial and insect defoliation on photosynthesis, growth and biomass allocation of *E. globulus* seedlings.

3.2. MATERIALS AND METHODS

3.2.1. Conditions of glasshouse

This glasshouse experiment was conducted at the Department of Primary Industry in Newtown (42° 51.22'S 147° 17.56'E). Glasshouse conditions were controlled to maintain day/night temperatures of 25/12°C with a relative humidity of approximately 45%.

3.2.2. Plant material

E. globulus seedlings of 0.01 m high raised in a commercial nursery were planted into 0.005 m³ pots filled with a mixture of pine bark (70%), washed sand (20%) and sieved loam (10%) (Horticultural Supplies, Brighton, Tasmania). The seedlings were grown in a shade-house for six months during which time they were watered to saturation three times daily, and fertilised twice with 15 g slow release nutrients (Osmocote® containing 16-3.5-10 of N-P-K) using soil surface application. After six months

seedlings averaged 0.5 m height and were re-potted into 0.009 m³ pots on 27th December 2006.

Twenty-four plants were selected on 9th February 2007 and stratified into eight block-groups of three plants based on similar height and leaf-area development. Overall, the average height of the seedlings was 0.84m within a range between 0.61 and 1.03 m. One plant from each group was allocated randomly to three treatments. The seedlings were transferred into a glasshouse and grown at 23°C, 45% relative humidity and 16 h photoperiod until the start of the experiment four days later.

3.2.3. Insect culture

Adults of *P. agricola* were collected in December 2006 from a *E. nitens* plantation near the Huon River, Tasmania (43.00°S, 146.49°E, elevation 100 m). Adults were collected from the field and maintained on fresh *E. globulus* juvenile foliage at 20°C and 16 h photoperiod. Eggs laid were transferred to young foliage and stored at 4°C to arrest development. Once sufficient eggs had been collected, eggs were reared at 20°C. By 10 days, the eggs had hatched and the larvae had passed through first and second instars and were transferred to treatment seedlings to feed while they passed through their final two instars before pupation.

3.2.4. Treatments and experimental design

Each seedling was divided initially into two equal zones by height and a third zone was added subsequently. Zone 1 (Z1) was totally separated from Z2 by an additional piece of netting. Zone 2 (Z2) included the defoliated top-half of the crown of the defoliated seedlings while Zone 3 (Z3) included the newly expanded crown that developed following treatment.

The three treatments applied to the eight seedlings in each treatment were no defoliation (control), insect defoliation and artificial defoliation. For the insect

defoliation, larvae of *P. agricola* (~100 per seedling) were distributed by hand onto sessile leaves throughout the Z2 canopy on 13th February 2007. The larvae fed for five days, when the visually-estimated target defoliation level of 25% was achieved; all larvae were then removed by hand. This estimated decrease in leaf area by insect defoliation was confirmed using the Crown Damage Index (Stone et al. 2003) as being 25%. For the artificial defoliation, sessile leaves and buds in Z2 were snipped manually with scissors. To simulate the spatial feeding pattern of *P. agricola*, both the complete and partial removal of leaf and bud material of similar age and location was required. The most practical way to proceed was through application of this treatment on the 15th February, that mimicked the first two days of insect defoliation, and again on the 18th February, that mimicked the additional three days of insect defoliation. The estimated level of artificial defoliation was confirmed using the Crown Damage Index as being 25%.

All seedlings were placed in individual cages (0.99 x 0.85 x 0.62 m) on the same day fitted with nylon netting that allowed penetration of ~65% incident light. On 18th February 2007, all cages were removed after the defoliation period ended. Measurements commenced on 26th February 2007 (day 0), 8 days after the defoliation treatments and were repeated on 5 days (26/02/2007), 17 days (15/03/2007), 23 days (21/03/2007) and 27 days (25/03/2007) after the application of the defoliation treatment.

3.2.5. Stem growth

Height (h) and over-bark basal stem diameter (d ; ~10 mm above the soil surface) of the seedlings were initially measured on 13th February 2007 and then on four occasions after the start of the experiment: 26/02/2007, 15/03/2007, 21/03/2007, 25/03/2007.

3.2.6. Gas exchange and chlorophyll concentration

Light-saturated photosynthetic rate (A_{\max}) and stomatal conductance (g_s) were measured on three undamaged and expanding leaves per seedling in Z2 using an open-flow infra-red portable gas-analysis system (CIRAS-1, PP Systems, Herts, UK) fitted with a light source providing a photosynthetic photon flux of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The ambient CO_2 concentration was maintained at 360 ppm. Measurements were made on four occasions (26/02/2007, 03/03/2007, 15/03/2007, 21/03/2007) using healthy and entire leaves between 1000 and 1400 h Australian Eastern Standard Time (AEST) concurrent with growth measurements. Air temperature and relative humidity within the chamber were between 23-25°C and 45-50%, respectively.

A SPAD chlorophyll meter (model 502, Konica-Minolta, Hong Kong, China) was used to estimate leaf chlorophyll index on the same three leaves used for the gas exchange measurements. Chlorophyll concentration (Chl) is strongly and linearly related to leaf chlorophyll index (see Pinkard et al. 2006c). In this study, chlorophyll index was converted to concentration using the following equation after confirming that this was not affected by defoliation treatments using a group linear regression analysis on Genstat10 (Quentin A.G., unpublished data).

$$\text{Ln[Chl]} = 1.49 \times \text{Ln[chlorophyll index]} - 6.49 \quad (\text{Eq.3.1})$$

3.2.7. Foliar chemistry

On 25th March 2007, 27 days after the application of the defoliation treatment, three leaves from Z2 (used for the final gas-exchange and chlorophyll meter measurements) and three leaves in the process of expanding from Z3 of each seedling were tagged and collected for analysis of chlorophyll, nitrogen (N), and phosphorus (P) concentrations, soluble sugars and starch contents. Specific leaf area (SLA) was expressed as the ratio of leaf fresh area and dry mass. Leaves were separated into two halves bilaterally. On the first half, total chlorophyll was extracted from leaf discs (9300 mm^2) using the acetone method (Agency USEP 1994). Chlorophyll content was calculated with the

equations of Arnon (1949). The remaining of the first half leaf was dried at 65°C for 48 h to constant weight and ground in a hammer mill. Samples were prepared for foliar nitrogen and phosphorus analyses using a continuous flow colorimetric auto-analyser (McLeod 1992). The other half of the three leaves was dried and ground similarly for colorimetric determination of total soluble sugar and starch content (Dubois et al. 1956; Buysse and Merckx 1993). Concentration of starch (glucose units) was calculated as described in Palacio et al. (2007). Total non-structural carbohydrate was the sum of soluble sugar and starch. Foliar contents (g m^{-2}) of total non-structural carbohydrate were calculated using their concentrations and specific leaf area.

3.2.8. Biomass harvesting

All seedlings were harvested on 25th March 2007, 27 days after the application of the defoliation treatment. Plant material from the three zones was partitioned into stem, branches and leaves and oven-dried to constant mass at 65°C (approximately 72 h) and weighed. Ten fully-expanded leaves per plant were collected from each crown position for analysis of specific leaf area (SLA) using a planimeter (Delta-T Device, Cambridge, U.K.). The samples were representative of the range of leaf sizes found on the seedlings. Analysis of biomass allocation and specific leaf area were determined on each individual dry mass component and components pooled together.

3.2.9. Data analysis

Increments of stem growth were calculated for height (h_{inc}) and diameter (d_{inc}) as the growth measured on day j less the growth initially measured. I calculated instantaneous water-use efficiency (WUE) expressed as the ratio of A_{max} and g_s , nitrogen-use efficiency (NUE) expressed as the ratio of A_{max} and nitrogen, A_{max} to chlorophyll ($A:\text{Chl}$) concentration ratio, stem mass ratio (%) expressed as the ratio of stem mass and total mass, branch mass ratio (%) expressed as the ratio of branch mass and total mass and leaf mass ratio (%) expressed as the ratio of leaf mass and total mass. The

effect of defoliation on growth and gas exchanges parameters were analysed by split plot for repeated measures. Two-way analysis of variance (ANOVA) was used to determine the effects defoliation treatments, crown zones and their interactions on biomass variables. The Genstat10 software (GENSTAT Committee 1989) was used for the ANOVA and determination of error bars using the least squares method (LSD).

3.3. RESULTS

3.3.1. Growth increment

Defoliation treatments significantly affected stem growth over the period of the experiment (Table 3.1). Following insect defoliation, h_{inc} and d_{inc} were reduced significantly compared to the control treatment ($P < 0.05$; Figures 3.1a, 3.1b). Although artificial defoliation had no significant effect on h_{inc} , it affected significantly d_{inc} ($P < 0.05$; Figure 3.1b).

3.3.2. Photosynthesis, stomatal conductance and water-use efficiency per unit leaf area

Both insect and artificial defoliations resulted in significant increases in A_{max} and g_s compared to the control (Table 3.1). Mean A_{max} was not significantly different between the two defoliation treatments although up-regulation was generally greater following insect than artificial defoliation ($P > 0.05$; Figure 3.2a). Larger values of g_s were found on 15/03/2007 and 21/03/2007, 17 and 23 days after the defoliation treatment, in insect-defoliated compared to artificially defoliated and control seedlings ($P < 0.05$; Figure 3.2b).

Table 3.1. Summary of significant ANOVA results showing degrees of freedom (*d.f.*), *F* and probability (*P*) for each analysis.

Analysis	Factor	<i>d.f.</i>	<i>F</i>	<i>P</i>
h_{inc} (m)	Treatment	2	5.64	0.011
	Date	3	244	0.001
	Date×Treatment	6	3.24	0.030
d_{inc} (mm)	Treatment	2	9.91	0.001
	Date	3	119	0.001
	Date×Treatment	6	3.97	0.005
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	2	14.2	0.001
	Date	3	3.65	0.023
	Date×Treatment	6	1.06	0.384
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	Treatment	2	3.98	0.026
	Date	3	13	0.001
	Date×Treatment	6	2.31	0.046
WUE ($\mu\text{mol mol}^{-1}$)	Treatment	2	0.18	0.834
	Date	3	4.24	0.010
	Date×Treatment	6	3.05	0.011
Chl (mg m^{-2})	Treatment	2	16.2	0.001
	Date	3	7.49	0.001
	Date×Treatment	6	5.95	0.001
$A:\text{Chl}$ ($\text{mmol g}^{-1} \text{s}^{-1}$)	Treatment	2	3.77	0.031
	Date	3	3.52	0.026
	Date×Treatment	6	0.65	0.655

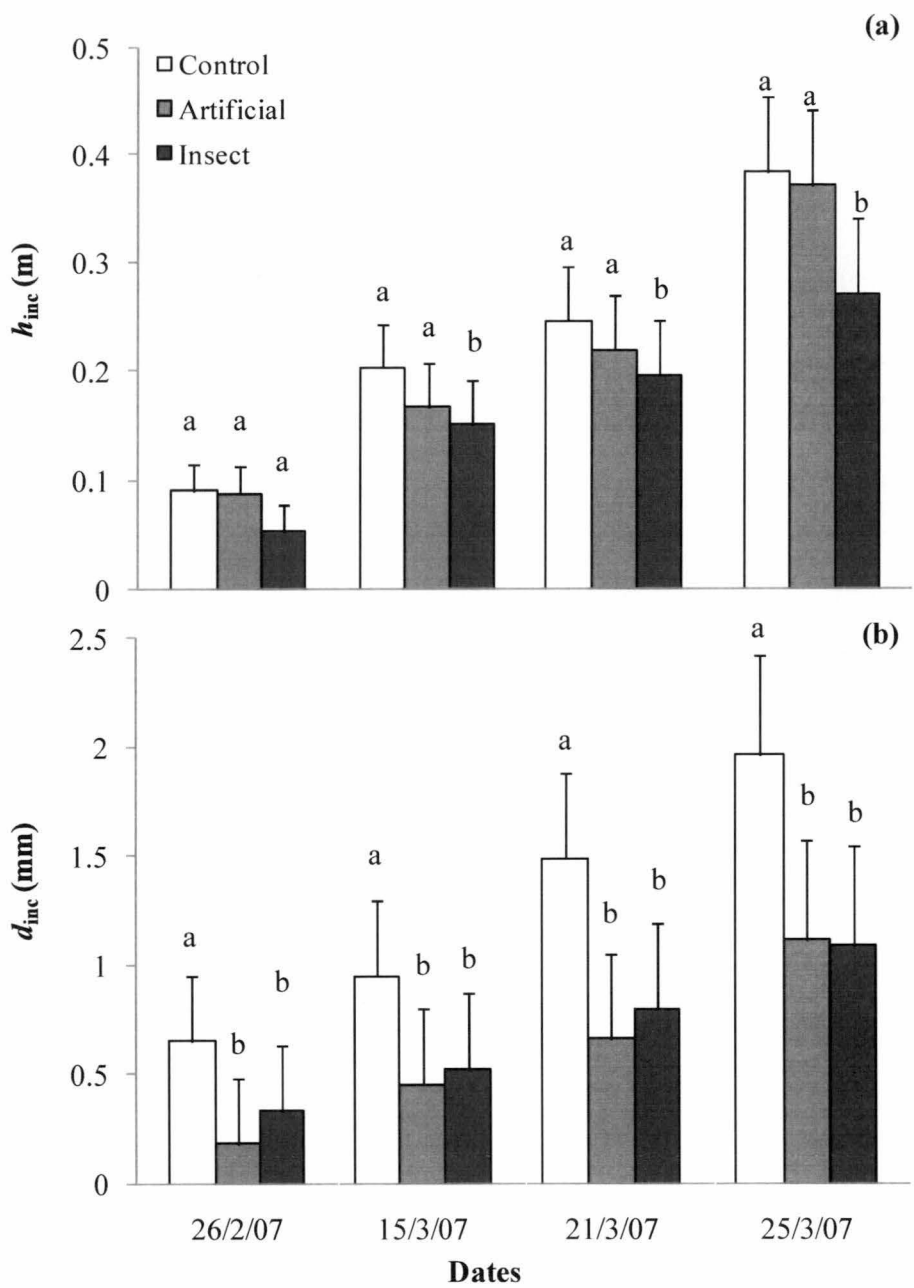


Figure 3.1. Mean increments of (a) height (h_{inc}) and (b) basal diameter (d_{inc}) measured on four occasions for control, artificial and insect. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

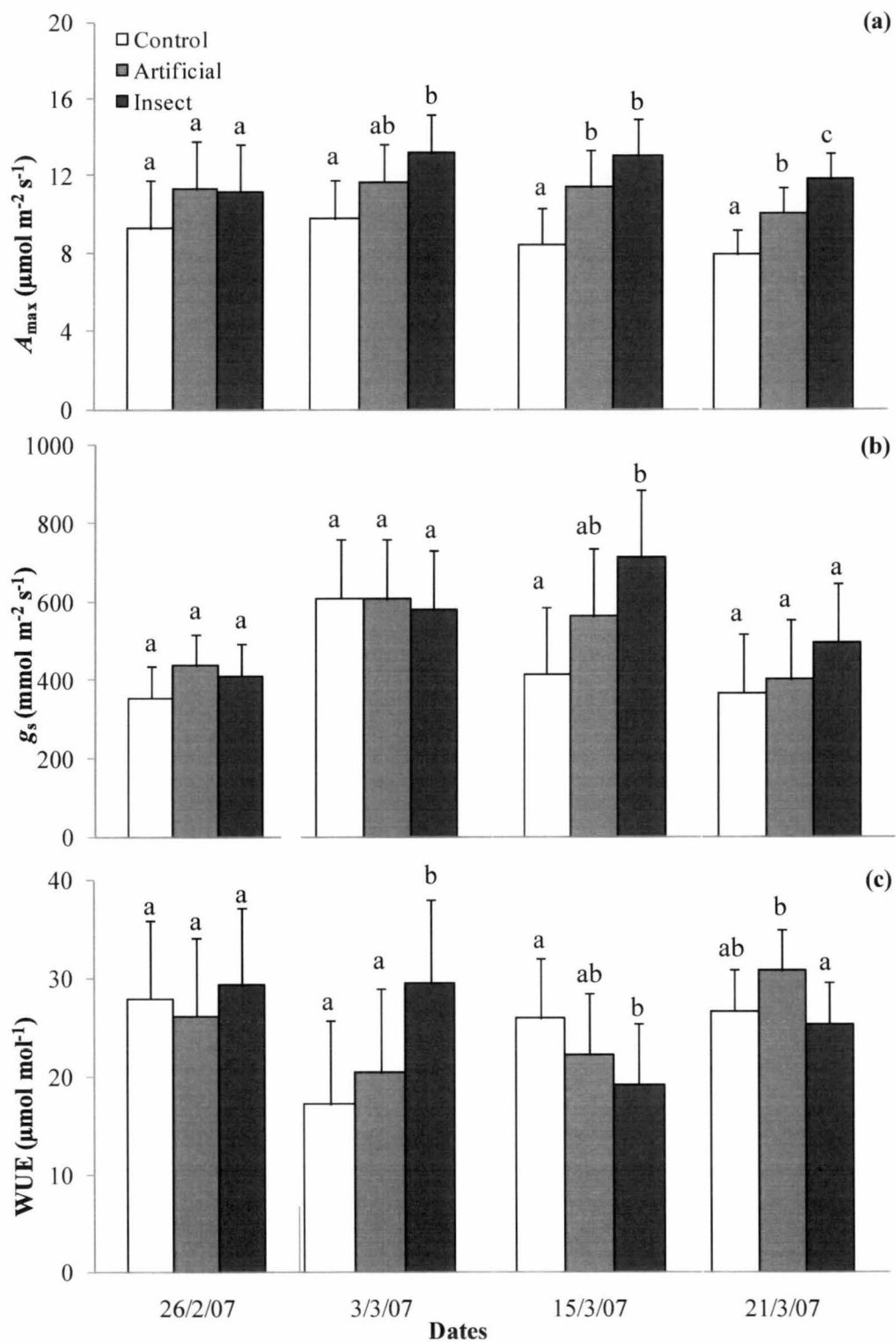


Figure 3.2. Mean (a) photosynthetic rate (A_{max}), (b) stomatal conductance (g_s), and (c) water-use efficiency (WUE) for control, artificial and insect defoliation treatments on four occasions after the defoliation. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

There was a significant effect by date and a significant date×treatment interaction on WUE (Table 3.1). By 03/03/2007, five days after the defoliation treatment, WUE of insect defoliated trees was significantly higher than that in the control and artificial defoliation treatments ($P<0.05$; Figure 3.2c), as a result of a larger increase in A_{\max} . WUE of the insect defoliated trees declined significantly by 15/03/2007, due to increased g_s . In contrast WUE of the control trees increased, in response to decreased g_s by the end of the study ($P<0.05$; Figure 3.2c). The effect of artificial defoliation on WUE was visible only 23 days later (21/03/2007) compared to the control ($P<0.05$, Figure 3.3).

3.3.3. Foliar chlorophyll concentration and A_{\max}

Defoliation treatments significantly affected chlorophyll and A_{\max} to chlorophyll ratio (Table 3.1). Foliar chlorophyll increased in both defoliation treatments (Figure 3.3a) and the largest increases were observed following insect defoliation ($P<0.001$, Figure 3.3a). In the control treatment, chlorophyll remained relatively constant throughout the experiment (Figure 3.3a). Ratios $A:\text{Chl}$ were generally higher in the insect-defoliated seedlings than in the artificial and controls, however this difference was only significant for insect and control treatments except on 21/03/2007 ($P<0.05$, Figure 3.3b).

3.3.4. Foliar nitrogen concentration and photosynthetic nitrogen-use efficiency

On 25/03/2007, 27 days after the defoliation treatment, insect defoliation resulted in higher nitrogen concentration ($P<0.05$; Figure 3.4a) in Z2, whereas artificial treatment did not differ from the control treatment ($P>0.1$; Figure 3.4a). In Z3, there were no differences between treatments ($P>0.1$; Figure 3.4a). In Z2, NUE did not differ between treatments. There was no significant relationship between A_{\max} and nitrogen. Foliar phosphorus was unaffected by both insect and artificial defoliation treatments (Figure 3.4b).

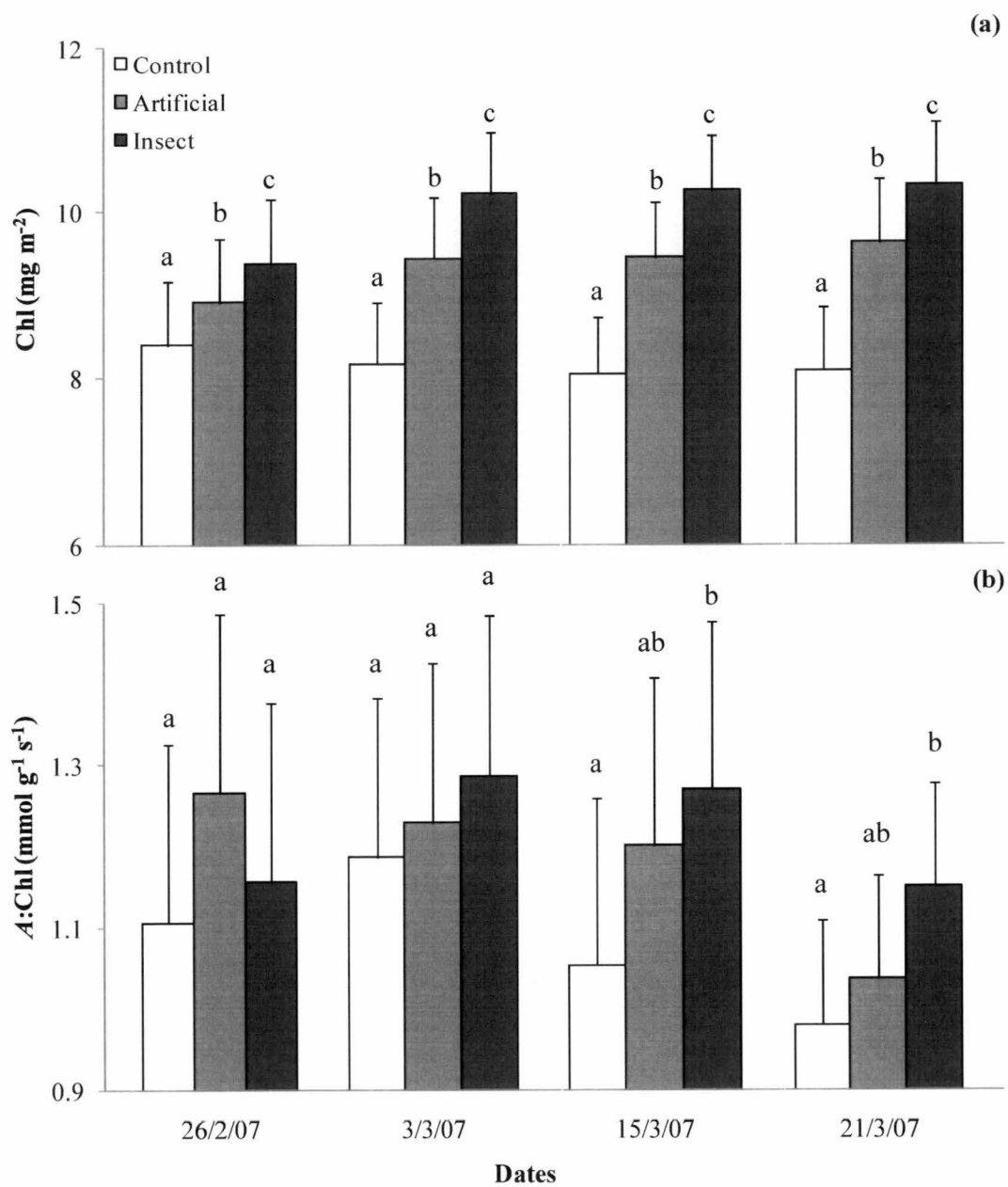


Figure 3.3. Mean (a) chlorophyll concentration (Chl) and (b) ratio between A_{max} and Chl ($A:Chl$) on four occasions following defoliation treatments. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

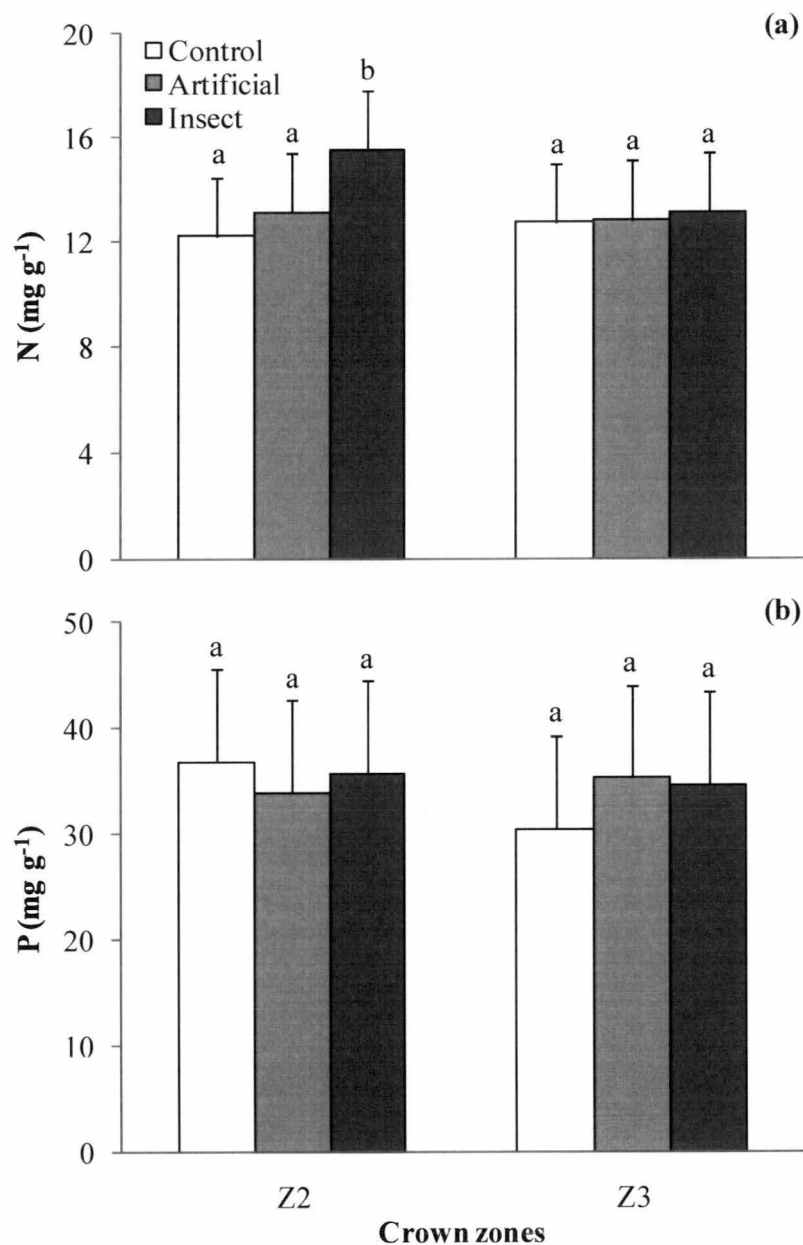


Figure 3.4. Means (a) nitrogen (N) and (b) phosphorus (P) concentrations 27 days after the defoliation in zones 2 and 3 (Z2, Z3). Error bars show least square differences ($\alpha<0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

3.3.5. Foliar non-structural carbohydrate content

Defoliation treatments had significant impacts on foliar total non-structural carbohydrate; however this response varied between zones. In Z2 there were no differences between control and artificial treatments, however insect defoliation resulted in a significant reduction in total non-structural carbohydrate (both soluble sugar and starch, $P < 0.05$; Table 3.2) compared to both control and artificial treatments. In Z3, where leaves were not affected directly by the treatment but expanded afterward, soluble sugars were reduced significantly in the artificial treatment compared to control treatment. Starch and total non-structural carbohydrate were significantly lower in both artificial and insect defoliation treatments ($P < 0.05$; Table 3.2), compared to controls.

3.3.6. Specific leaf area and biomass partitioning

Zone had a significant effect on SLA ($P < 0.001$) with the highest increases occurring in Z3 and the lowest in Z1 (Figure 3.5). Significant increase in SLA of Z2 due to insect defoliation (25%) was observed ($P < 0.05$; Figure 3.5). Both defoliation treatments also caused 15% increase in SLA of Z3 ($P > 0.05$; Figure 3.5). Both artificial and insect defoliation significantly reduced total biomass by 28.3% and 16%, respectively, compared to the control treatment ($P < 0.05$). Artificially defoliated seedlings allocated less branch biomass in Z1 but more in Z2 compared to the control seedlings ($P > 0.1$; Table 3.3). They also allocated more biomass to the stem in Z3 than the control ($P > 0.1$, Table 3.3), whereas the seedlings in the insect treatments allocated less than the controls ($P > 0.1$, Table 3.3). However, insect defoliated seedlings allocated significantly more biomass to the branch in Z2 than the control seedlings ($P < 0.05$; Table 3.3). They also produced more foliage in Z3 than the control seedlings ($P > 0.1$), whereas seedlings in the artificial treatment produced relatively less foliage than the controls (Table 3.3).

Table 3.2. Mean foliar non-structural carbohydrates contents for control and defoliated seedlings across zones 2 and 3 (Z2, Z3) 27 days after the defoliation. LSD homogeneous groupings are identified by letters ($\alpha=0.05$). Values were taken from Genstat.

	Control	Artificial	Insect
Content in Z2 (g m^{-2})			
Soluble sugars	4.65 (± 0.82) a	4.30 (± 0.47) a	2.72 (± 0.29) b
Starch	1.83 (± 0.28) a	1.76 (± 0.28) a	0.99 (± 0.19) b
Non-structural carbohydrates	6.48 (± 0.98) a	6.06 (± 0.74) a	3.7 (± 0.46) b
Content in Z3 (g m^{-2})			
Soluble sugars	4.7 (± 1.10) a	2.79 (± 0.32) b	3.45 (± 0.89) ab
Starch	1.72 (± 0.57) a	0.77 (± 0.15) b	0.64 (± 0.11) b
Non-structural carbohydrates	6.43 (± 1.65) a	3.56 (± 0.46) b	4.09 (± 0.99) b

Table 3.3. Mean values (\pm SE) of above-ground (stem, branch, leaf) mass ratio across each crown zone and for the three crown zones pooled together for control and defoliated seedlings 27 days after the defoliation. LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

Crown zones	Treatments	Stem mass ratio	Branch mass ratio	leaf mass ratio
Zone 1	Control	64.7 (± 7.8) a	6.9 (± 2.7) a	28.4 (± 5.2) a
	Artificial	63.6 (± 6.1) a	5.6 (± 1.9) a	30.8 (± 4.9) a
	Insect	57.5 (± 5.3) a	8.3 (± 2.2) a	34.2 (± 3.6) a
Zone 2	Control	30.5 (± 4.6) a	13.9 (± 1.2) a	55.6 (± 3.6) a
	Artificial	28.5 (± 3.3) a	16.1 (± 1.5) ab	55.4 (± 2.7) a
	Insect	27.6 (± 2.2) a	19.1 (± 1.1) b	53.3 (± 2.6) a
Zone 3	Control	14.3 (± 0.9) a	18.2 (± 0.5) a	67.5 (± 0.9) a
	Artificial	15.6 (± 0.7) a	18.4 (± 1.5) a	66.0 (± 1.8) a
	Insect	12.0 (± 1.0) a	18.9 (± 1.4) a	69.1 (± 1.0) a
Whole tree	Control	36.5 (± 5.2) a	13.0 (± 1.4) a	50.5 (± 4.0) a
	Artificial	35.9 (± 4.8) a	13.4 (± 1.5) a	50.7 (± 3.6) a
	Insect	32.4 (± 4.4) a	15.4 (± 1.4) a	52.2 (± 3.3) a

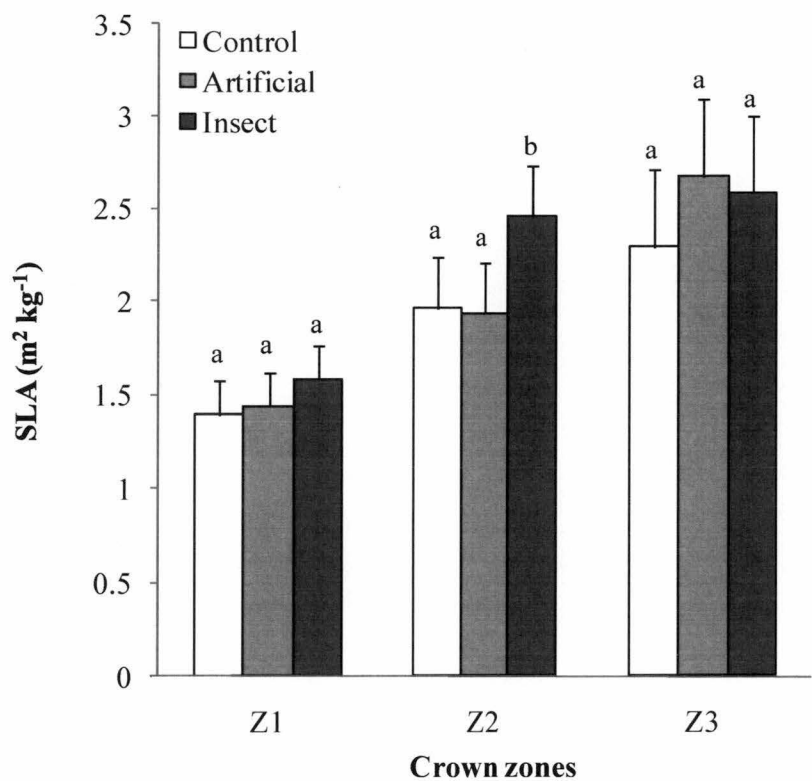


Figure 3.5. Mean specific leaf area (SLA) for control and defoliated seedlings 27 days after defoliation. Error bars show least square differences ($\alpha=0.05$). LSD homogeneous groupings are identified by letters ($\alpha=0.05$).

3.4. DISCUSSION

In various studies that measured growth rate, the type of defoliation had contrasting effects on height growth component. For example, Chen et al. (2002) reported that artificial defoliation of Douglas-Fir had a significant negative effect on height and diameter growth, whereas defoliation by western spruce budworm (*Choristoneura occidentalis* Freeman) had a significant negative effect only on diameter growth. In contrast, other studies with sawflies (*Neodiprion sertifer* (Geoff.) and *N. Gillettei* (Rohwer)) found significant reductions in height and diameter growth increment following both insect and artificial defoliation treatments (Britton 1988; Sanchez-Martinez and Wagner 1994). In *E. globulus*, insect defoliation had a significant adverse impact on both height and diameter growth, whereas artificial defoliation only affected the diameter growth. These results suggest that artificial defoliation underestimated the impacts of insect defoliation on height growth. The differences in the results with the previous studies may have been in the methods used to simulate the natural defoliation. However, it is generally difficult to tease out the method used for the artificial treatment and to know how well it mimics the insect activity. In Chen et al. (2002), the difference in the growth response between the two types of defoliation imposed to describe the treatments and special attention was drawn towards the removal of buds. They found that artificial defoliation removed buds completely preventing leader growth, whereas some buds were partially consumed by budworm larvae allowing shoot elongation.

The finding suggests that the growth of *E. globulus* seedlings can be affected by a low level, single event of insect herbivory. Damage to buds following herbivory affects growth and patterns of resource allocation within trees (Haukioja et al. 1990). Buds determine patterns of growth that are exerted through the suppressive effects of dominant apical meristems upon other tissues, and because they represent ‘sinks’ of the previous photosynthetic products (Honkanen et al. 1994), as well as being the source of both foliage and reproductive structures. Despite the best endeavours to simulate the spatial pattern of insect defoliation in the artificial treatment, the latter may have caused greater bud damage and hence had stronger effect on apical

dominance and height growth. Thus the result obtained in such experiments may be as much a consequence of the relativities of bud removal as of leaf area removal.

Up-regulation of photosynthetic rates in the remaining leaves following natural and artificial defoliation has been observed in many tree species (Reich et al. 1993; Vanderklein and Reich 1999; Chen et al. 2001) including in eucalypt species (Pinkard et al. 1998; Pinkard et al. 2007; Turnbull et al. 2007). Up-regulation is likely to play an important role in minimising the impact of defoliation on growth (e.g. Heichel and Turner 1983; Ovaska et al. 1993b; Reich et al. 1993; Vanderklein and Reich 1999; Chen et al. 2001). For example, Pinkard and Beadle (1998a) reported that removal of 50% of leaf area of *E. nitens* seedlings had no impact on height growth 8 weeks later, which they related to enhanced photosynthetic rates (Pinkard et al. 1998) and changes in patterns of carbon allocation to favour leaf area development (Pinkard and Beadle 1998a). However there have been no studies that I was aware of that have compared physiological responses in artificially and naturally defoliated trees. Our results indicated that for a similar pattern of damage, the photosynthetic up-regulation in response to insect defoliation was 15% larger compared to artificially-defoliated seedlings, although this response varied over time (Figure 3.2).

Artificial defoliation of young *E. globulus* in plantations substantially increased chlorophyll concentration of the remaining leaves (Pinkard et al. 2007). A similar observation was made in this study with seedlings, though insect defoliation had a stronger effect (Figure 3.3a). Changes in A_{\max} following partial defoliation could have been attributed to an increase in chlorophyll concentration in the remaining leaves because an increase in chlorophyll concentration leads to an increase in the amount of photosynthetic enzymes (Trumble et al. 1993). Although partial defoliation may result in higher foliar N concentration and photosynthetic activity (Trumble et al. 1993), there was no evidence in this study of photosynthetic enhancement related to foliar nitrogen. Numerous studies have similarly concluded that changes in nitrogen following defoliation were not responsible for enhanced photosynthesis (Ovaska et al. 1993b; Reich et al. 1993; Turnbull et al. 2007), although others report a positive correlation (Hoogesteger and Karlsson 1992).

The photosynthetic response was rapid, a finding that substantiates the view that small plants generally respond more quickly to defoliation than larger plants (Heichel and Turner 1983), and that the response is shorter (Reich et al. 1993). Seedlings have a smaller capacity for storage of assimilates than larger plants. Thus, following loss of photosynthetic area, the demand on stored carbon reserves is likely to be intense (Cesaroli et al. 2004) and result in relatively high consumption or export of assimilates. The present study suggests that insect defoliation increased export of sugars from the leaf (Table 3.2). Additionally, insect defoliation resulted in higher branch biomass than artificial defoliation in Z2 ($P>0.05$; Table 3.2). Based on the source-sink hypothesis (Honkanen et al. 1999), damage by herbivory modifies the strength of either pre-existing sink or newly formed sinks. In the present study, insect defoliation altered the ability of new shoots to draw resources to maintain stem growth, although it increased sink strength of directly damaged leaves. However, the suppression of apical dominance by the insect defoliation induced significant increases in allocation of biomass to the branches in the damaged crown zones (Z2). In artificially-defoliated seedlings, sufficient carbon export to the shoot tip allowed to maintain similar rates of shoot elongation and partially stimulate branching in Z2. This is an interesting finding and further studies would be necessary to explain how the changes in apical dominance and branching pattern have a role in growth compensatory response by *E. globulus* seedlings in response to insect defoliation.

Investigators have difficulty matching the response of host plants to herbivory with artificial defoliation treatments, even when the amount of tissue loss is tightly controlled. It is also suggested that the host responses at the feeding site is more related to a phytotoxic response to herbivory than salivary enzymes injected by insects (Mithöfer et al. 2005). Components of insect saliva, such as oxidase and sucrase, also affect host response (Steinbauer et al. 1997). For example, *Amorbus obscuricornis* (Westwood) (Heteroptera: Coreidae) caused wilting and necrosis of apical shoots of eucalypt seedlings which led to a loss of apical dominance and height growth as well as stimulation of lateral bud development (Steinbauer et al. 1997). In this study, the browsed leaves and shoots may have been biochemically more reactive to particular salivary components of *P. agricola* than to cutting with scissors, a factor that may have

contributed to reduced apical dominance and the significant reduction in height growth observed following insect defoliation.

The results proved clearly the first hypothesis that the seedlings responded to both defoliation treatments with measures showing significant differences from control. Also, there was strong evidence that support the second hypothesis that the direction of response to artificial and insect defoliation was very similar. However, the influence of differential magnitude of the responses is more difficult to ascertain. I conclude that artificial defoliation may not accurately reflect the full strength of effects from insect defoliation, and caution must be exercised in extrapolating results of simulated herbivory experiments beyond the level of the individual effects to more complex ecological interactions (Hjältén 2004). Nevertheless, artificial defoliation still has its place in the toolbox of the ecologists. Like previous studies, the findings suggest that simulated herbivory provides a useful a priori basis for acquiring fundamental insights on the effects of insect defoliation (e.g. *Eucalyptus regnans* F.Muell, Candy et al. 1992). With the issue of injury guilds (see chapter 1), the results from this study potentially are important for integrated pest management (IPM) applications. One of the key requirements for developing multiple-species IPM is that the pest species must produce a similar type of injury. Based on results from previous studies, homogeneities in photosynthetic responses occur between different defoliators and different plant species. This homogeneity can then be used to determine a whole plant response based artificial defoliation for multiple pests. Because there were no significant differences between insect and artificial defoliation, it seems possible to develop a generalised primary physiological response to defoliation for *E. globulus*. Also, simulating herbivory may facilitate the modelling of growth responses (Watt et al. 2007) and help the development of sophisticated tools for biological control (Conrad and Dhileepan 2007). The appropriateness of using simulated herbivory largely depends on the question addressed and hypothesis tested (Hjältén 2004). For future research, it would be valuable to look at how the pattern of response of a plant to pest attack is influenced by the kind of damage inflicted (defoliating insect, sap sucking insect, fungi, artificial) and the speed at which leaves are removed (instantaneous versus long-term defoliation).

Chapter 4: Growth, Biomass Allocation and Carbohydrate.

4.1. INTRODUCTION

Although the effect of defoliation on plant performance in the first instance appears to be detrimental, many species eventually respond with higher rates of plant growth than those prior to defoliation (Bassman and Dickmann 1982; McNaughton 1983; Belsky 1986; Haukioja and Koricheva 2000). A range of mechanisms is first observed in defoliated plants (Prins and Verkaar 1992) that include reductions in growth rate and total biomass (Piene and Little 1990; Ovaska et al. 1993a; Reich et al. 1993; Sanchez-Martinez and Wagner 1994; Lyytikainen-Saarenmaa 1999; Vanderklein and Reich 1999; Chen et al. 2002; Eyles et al. 2009b), changes in shoot:root ratio (Eyles et al. 2009b), modified branching pattern (Schat and Blossey 2005), decreased or increased photosynthetic activity (Ovaska et al. 1993a; Reich et al. 1993; Kolb et al. 1999; Vanderklein and Reich 1999; Pinkard et al. 2007; Turnbull et al. 2007), changed foliar chemistry (Turnbull et al. 2007), reduced inflorescence production (Schat and Blossey 2005), and changes in the levels of chemical defence (Boege 2005). Plant response depends also on defoliation intensity (Piene and Little 1990; Reich et al. 1993; Kolb et al. 1999), timing of damage (Honkanen et al. 1994), defoliation history (Vanderklein and Reich 1999), plant genotype (Sanchez-Martinez and Wagner 1994), tree species (Vanderklein and Reich 1999), the plant parts removed (Haukioja et al. 1990; Honkanen et al. 1994) and environmental factors (Honkanen et al. 1999; Eyles et al. 2009b).

In the above studies, small plants or juvenile trees (aged < 3 yr) were used. Young trees are often relatively more responsive to defoliation (Boege 2005) and logistically easier for conducting research than older trees. Such an approach fails to account for ontogenetic changes in function with increasing plant size. For example, fast-growing young eucalypts and other species initially display exponential stem growth that is associated with a large capacity for new leaf development. With increasing age and

size, the carbon–nutrient balance, storage capacity, and shoot:root ratio increase, while growth rates and metabolic activity decrease (Medhurst et al. 1999). Moreover, post canopy-closure trees have a relatively stable leaf index area, and therefore potentially less capacity to respond to defoliation through leaf area renewal.

Even less is known about the extent to which defoliation affects crown architecture of older trees (Ericsson et al. 1980; Krause and Raffa 1996; Buck-Sorlin and Bell 2000; Puntieri et al. 2006), their capacity to reallocate biomass (Krause and Raffa 1996; Pinkard and Beadle 1998a) and resources (Ericsson et al. 1980; Boege 2005). The reasons for this deficiency of information are probably associated with their high architectural complexity (Pinkard and Beadle 1998b; Puntieri et al. 2006), slow response and long recovery time after a defoliation event (Krause and Raffa 1996; Haukioja and Koricheva 2000; Puntieri et al. 2006). For instance, in ten-year-old *Pinus resinosa* Hook., stem radial growth was immediately reduced by defoliation but there was no significant reduction in height growth until 14 months after the event (Krause and Raffa 1996).

Many defoliation studies target small trees as model systems (e.g. Loch and Floyd 2001). A reason for using small trees is that they are logistically easier to work with and allow adequate replication within treatments. Substantial defoliation also occurs in older trees (e.g. Candy et al. 1992). Little is known about the physiological responses of large trees to defoliation. The present study investigated four-year-old, 13-m height *E. globulus* trees in a closed canopy stand. Undertaking a wide range of physiological measurements on trees of this stature introduces logistical constraints on experimental design and limits the capacity for replication. Three undefoliated and three defoliated trees were measured over a period of 322 days and the results are presented here and in chapters 5 and 6. The small size of the sample inevitably influenced the power of the statistical analysis and this is likely to affect the statistical significance of measured differences between treatments. Because of this some reliance is placed on marked differences in biological trends to make statements about treatment effects. In this study, the impact of artificial defoliation was assessed on:

1. subsequent plant growth, testing the hypothesis that defoliated *E. globulus* maintains stem growth at the expense of other biomass pools;
2. changes in crown architecture, testing the hypothesis that loss of leaf area results in accelerated rates of new leaf development; and
3. utilisation and storage of non-structural carbohydrates among organs, testing the hypothesis that current carbohydrates are used to replace foliage lost to maintain growth.

4.2. MATERIALS AND METHODS

4.2.1. Defoliation treatment

When the trees were four-years old, an experiment was conducted on six trees within a single plot (15 × 15 m) of the existing experimental design (O’Grady et al. 2006). The six trees had a range of sizes of 13.1 to 13.2 m in height and 115 to 168 mm in diameter at breast height (1.3 m).

Two treatments, defoliated and undefoliated (control), were allocated randomly to three trees each. Starting late winter, on the 29th August 2006 (day 0), and using an elevated platform, I removed all leaves using long-nosed secateurs, excluding the apical buds, from the top 50% of crown length of the three trees allocated to the defoliation treatment. Prior to defoliation, the diameter of all branches on the tree was measured approximately 100 mm from the junction with the stem. Based on allometric equations developed by O’Grady et al. (2006) between branch over-bark diameter and leaf area, I estimated that approximately 45% of leaf area was removed.

4.2.2. Growth responses

Total height (h) and diameter at breast height (d) of the trees were measured one day before the defoliation and at seven times following defoliation (Oct-06; Nov-06; Dec-06; Jan-07; Mar-07; May-07; Jul-07).

4.2.3. Biomass harvesting

To investigate the effect of defoliation 322 days after treatment application, the six experimental trees were harvested destructively for analysis of above- and below-ground biomass. Above-ground harvesting was undertaken at the end of the growing season in mid-winter (17/07/07).

For each tree, the crown was divided into three height zones to allow distinction of within-tree variation, particularly as a function of old and new growth. These zones were: (a) lower crown zone (Z_L) = the non-defoliated half of the initial live crown length; (b) middle crown zone (Z_M) = the initial tree height minus the lower crown zone; and (c) upper crown zone (Z_U) = all new growth following treatment application. Trees were felled and measured for total height and height to the base of each crown zone. Stem diameters were then measured at the crown base (0.1 m above ground level), 1.3 m and at the base of each crown zone. For each zone, diameter of all live branches was measured at 100 mm from the stem. This point was marked for future reference during laboratory analysis of branch traits. Five branches which represented the range of branch diameters were selected in each zone (e.g. Pinkard and Beadle 1998a; Medhurst et al. 1999). The five branches were excised at the stem junction, labelled and placed in plastic bags for transport to the laboratory. The branches were stored at 4°C for a maximum of two days before processing.

Laboratory procedures of the branches described in Pinkard and Beadle (1998a), and Medhurst et al. (1999). A random stratified sample of 10 fresh leaves per branch per zone was collected for analysis of mean leaf size (mm^2) and specific leaf area (SLA). Leaf areas (A_L) as mean leaf sizes were measured using a planimeter (Delta-T Devices,

Cambridge, U.K.). After area measurement, the 10-leaf sample was also dried to constant weight at 65°C.

A cross-section of the stem was cut at 0.1 m, 1.3 m, and from the base of the three crown zones to determine sapwood area. The cross-section was stained with 0.2% dimethyl yellow in ethanol solution to reveal existence of heartwood. No heartwood was detected. The cross-section were labelled and placed in sealable plastic bags for transport to the laboratory. Determination of the under-bark cross-sectional area of sapwood at 0.1 m ($A_{S0.1}$), 1.3 m ($A_{S1.3}$) and from the base of the three crown zones followed the procedures described in Medhurst et al. (1999).

Stem wood density (ρ) was measured using method described in Thomas et al. (2006). Bark was removed from stem discs for each crown zone. Each disc was immersed in a container of distilled water, just under the surface, of known mass on a digital balance. Volume of fresh wood was equal to weight of water displaced. Dry mass of the samples was determined after oven drying for 48 h at 65°C, and wood density calculated as dry mass per unit of volume. After drying, samples representing the wood formed for the last 322 days were removed from the discs taken at 1.3 m. These samples were re-immersed into distilled water until saturation. Wood density for the growth period (ρ_{322}) was determined.

Below-ground harvesting was performed two weeks after the above-ground harvesting. For each tree, rootball and bulk root were excavated at set radial distance of approximately 2 m and at a depth of 2 m. Harvesting involved carefully removing by hand the sand around all vertical and lateral roots. Root samples (>5 mm in diameter) were placed into plastic bags and stored at 4°C. Coarse root biomass (W_{CR}) was separated into five root classes: (a) 5-10 (C_{5-10}); (b) 11-15 (C_{11-15}); (c) 16-20 (C_{16-20}); (d) > 21 ($C_{>21}$) mm diameter; and rootball, and oven dried at 65°C to constant dry weight. Prior to drying, the rootball was chopped into smaller pieces to facilitate the drying process. Following drying, all coarse root samples were weighed. Total coarse root biomass was calculated by summing dry mass across each diameter classes including the rootball.

4.2.4. Non-structural carbohydrate analysis

Biomass of each component was pooled in each crown zone and sub-sampled for total non-structural carbohydrate analysis. Starch and soluble sugars concentration were extracted by the Palacio et al. (2007) method. Soluble sugars were extracted from 50 mg of dried tissue with a solution of 10 mL of 80% (v/v) ethanol in a 60°C water bath. Starch and any remaining complex sugars were extracted from the resulting residue with 0.2 M sodium acetate and 0.5% amyloglucosidase (Fluka-10115). The concentrations of soluble sugar and starch were analysed using the Dubois et al. (1956) method as modified by Buysse and Merckx (1993), using a phenol/sulphuric acid colorimetric assay. Absorbance was read at 490 nm on a spectrophotometer (UV-VIS). A glucose solution was used for the standard. Concentration of starch was referred to in glucose units (Palacio et al. 2007). Total non-structural carbohydrate values were obtained by addition of soluble sugar and starch values.

4.2.5. Data analysis

Mean h_{inc} (m) and d_{inc} (mm) increments were calculated for each measurement date. The effect of defoliation on growth increment was analysed by repeated measures ANOVA. Stem volume (V) was determined, using crown zone length (h_z) and sapwood area at each end of the zone with equations below (A_{SZi}). The stump (between 0.1 and 1.3 m) was treated as a cylinder (Eq. 4.1), the tip was treated as a cone (Eq. 4.2), and the remaining crown zones were treated as quadratic frustums using Smalian's formula (Eq. 4.3):

$$V_1 = A_{S0.1} \times h_{0.1} \quad \text{Eq. 4.1}$$

$$V_2 = (A_{SZu} \times h_{zu}) / 3 \quad \text{Eq. 4.2}$$

$$V_3 = \sum_{i=2}^3 [(A_{SZi} + A_{SZi+1}) \times h_i] / 2 \quad \text{Eq. 4.3}$$

where $A_{S0.1}$ was the basal sapwood area; $h_{0.1}$ was the length of the stump; A_{SZu} was the sapwood area at the base of Z_U ; h_{Zu} was the length of Z_U ; A_{SZl} was the area of the large end of the crown zone i ; A_{SZi+1} was the area of the small end of the crown zone i and h_i was the length of the crown zone i . Finally the total stem volume was calculated as the sum of the three equations (Eq. 4.4):

$$V = \sum_{i=1}^3 V'_i \quad \text{Eq. 4.4}$$

Based on previous allometric equations (O'Grady et al. 2006), I estimated initial leaf area (A_L) and branch length (L_{Br}) and under-bark branch diameter (d_{ub}) prior to defoliation, using diameters (d_{Br}) of all branches initially measured in Z_L and Z_M . At the harvest, allometric equations were developed from the harvested branches between d_{Br} , and A_L , L_{Br} , d_{ub} , branch dry mass (W_{Br}) and foliage dry mass (W_F). The equations were modelled using a power function (Causton 1985). Those equations were then applied to all branch diameters measured over the entire stem to estimate A_L , L_{Br} , d_{ub} , W_{Br} and W_F per individual branch, and summed per zone. Branch cross-sectional area at 100 mm from stem (CSA) was calculated from d_{ub} measures assuming radial symmetry.

Stem biomass (W_S) was calculated from ρ and V . Above-ground woody biomass (W_w) was calculated as the sum of the W_S and W_{Br} . Whole-tree stem, branch, leaf and wood biomass were calculated by summing individual organ dry mass (kg biomass tree⁻¹). Above-ground biomass (W_{above}) was calculated as the sum of stem, branch and leaf biomass. Whole-tree biomass (W_{TREE}) was calculated as the sum of above- and below-ground biomass. The ratio of foliage to branch dry mass (FWR) were calculated for each crown zone. Shoot: root ratio (SRR) was calculated by dividing W_{above} and W_{CR} .

Differences between treatments were estimated using analysis of variance (ANOVA). The ANOVA procedure was used to determine the effects of defoliation on leaf area growth, stem and branch variables, biomass partitioning and allocation, and carbohydrate concentration at the end of the experiment. The analysis incorporated the effects of defoliation treatment and crown zone or root diameter class. The data were checked for normality and homogeneity of variances and if necessary, the values were

transformed to satisfy the assumption of ANOVA. All analyses of variance and regression were performed using Genstat10 software (GENSTAT Committee 1989).

4.3. RESULTS

4.3.1. Stem growth

Mean height increment was unaffected by defoliation over the duration of the experiment ($P>0.1$; Figure 4.1a). Diameter increment of defoliated trees was significantly reduced 155 days (mid-summer) after the treatment application, and this difference was maintained until spring ($P<0.1$; Figure 4.1b). By the time of the harvest, the defoliation treatment had less influence ($P>0.1$; Figure 4.1b); suggesting that negative effect of defoliation on diameter increment was short-term in nature. A_S and V_S were unaffected by treatment ($P>0.1$; Table 4.1). Wood density at 0.1 m ($\rho_{0.1}$) and 1.3 m ($\rho_{1.3}$) height was unaffected by treatment (Table 4.1). Wood density of the stem growth increment (ρ_{322}) was also unaffected by treatment ($P>0.1$; Table 4.1).

4.3.2. Leaf area development

At a whole tree level, SLA of defoliated trees was 33% larger than that of the undefoliated trees ($P=0.05$; Table 4.1) and defoliated trees also displayed a larger SLA than undefoliated trees in each crown zone ($P>0.05$; Figure 4.2a). There were no significant differences in leaf size between treatments ($P>0.1$; Table 4.1). At the end of the experiment defoliated trees still had 57% of the leaf area of undefoliated trees ($P>0.1$; Table 4.1), due to 42% less A_L in the defoliated zone (Z_M) ($P<0.1$; Figure 4.2b). In Z_L , A_L of undefoliated trees was lower by 5 m² compared to defoliated trees ($P>0.1$; Figure 4.2b). However, in Z_U , defoliated trees produced approximately half of the leaf area produced by the undefoliated trees ($P>0.1$; Figure 4.2b).

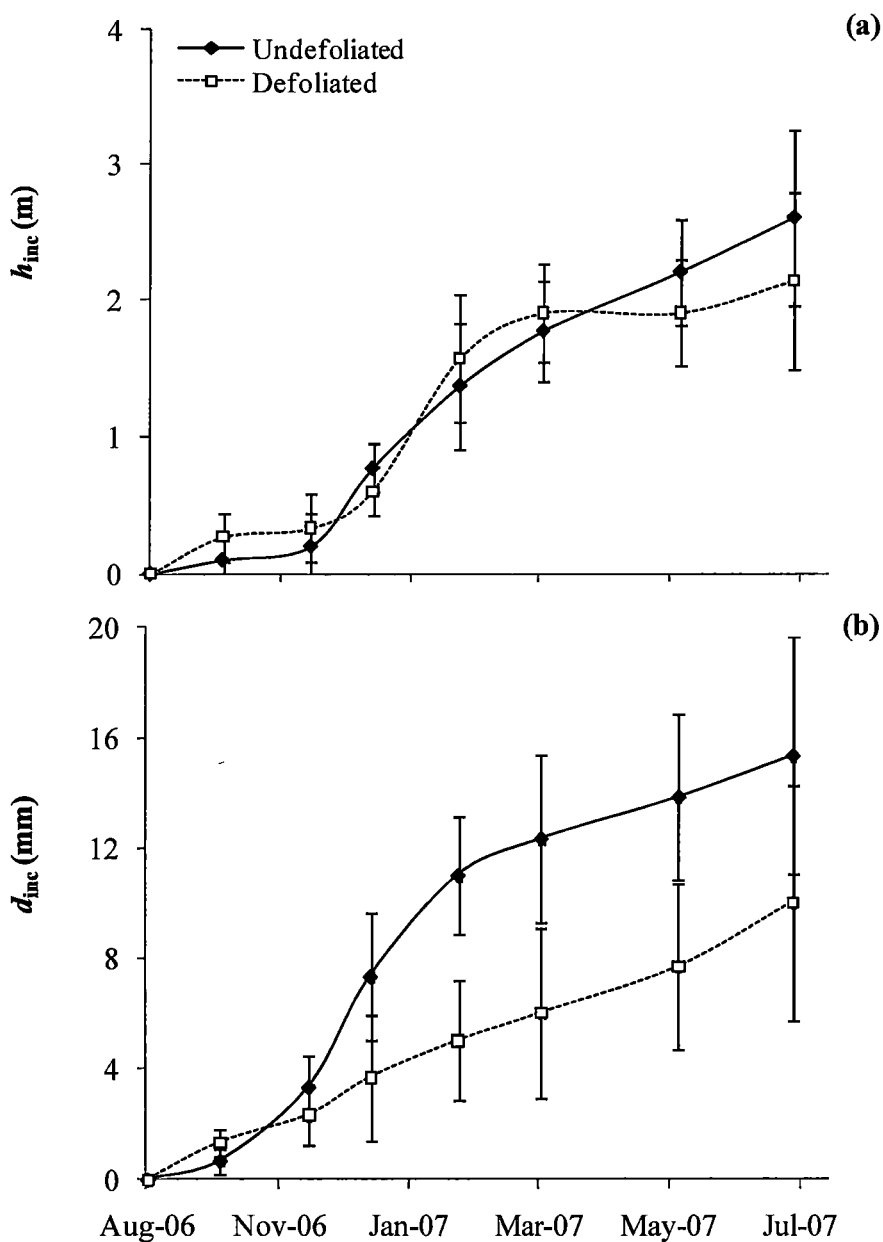


Figure 4.1. Mean (a) height (h_{inc}) and (b) diameter at 1.3 m height (d_{inc}) increment of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on eight occasions after defoliation. Errors bars represent standard errors ($\alpha=0.05$).

Table 4.1. Effects of defoliation on whole-tree mean stem growth, leaf area and branch variables of undefoliated and defoliated *E. globulus* trees 322 days after treatment imposed. Values are means of three replicates with SE in parentheses. ^ indicates whole-tree stem volume.

Variables	Undefoliated	Defoliated	P values
Height increment (h , m)	2.76 (0.41)	2.13 (0.47)	0.883
Diameter increment at 1.3 m (d , mm)	15 (4)	10 (2)	0.159
Leaf size (mm^2)	43 (0.2)	41 (0.2)	0.429
Specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$)	3.6 (0.2)	4.8 (0.1)	0.050
Leaf area (A_L , m^2)	47 (5)	36 (4)	0.203
Sapwood area at 0.1 m ($A_{S0.1}$, m^2)	0.021 (0.003)	0.018 (0.003)	0.399
Sapwood area at 1.3 m ($A_{S1.3}$, m^2)	0.017 (0.004)	0.013 (0.002)	0.380
Wood density at 0.1 m ($\rho_{0.1}$, kg m^{-3})	0.50 (0.02)	0.50 (0.03)	0.919
Wood density at 1.3 m ($\rho_{1.3}$, kg m^{-3})	0.49 (0.02)	0.48 (0.02)	0.703
Wood density of the growth increment (ρ_{322} , kg m^{-3})	0.50 (0.01)	0.48 (0.1)	0.833
Stem volume (V_S^{\wedge} , m^3)	0.40 (0.10)	0.39 (0.06)	0.920
Number of branches	91 (4)	79 (9)	0.289
Branch diameter (d_{Br} , mm)	1.2 (0.1)	1.4 (0.1)	0.494
Branch length (L_{Br} , m)	1.6 (0.01)	1.6 (0.08)	0.558
Foliage to branch wood ratio (FWR)	0.9 (0.2)	0.7 (0.2)	0.504
Shoot:root ratio (SRR)	5.4 (0.6)	6.8 (1.3)	0.390

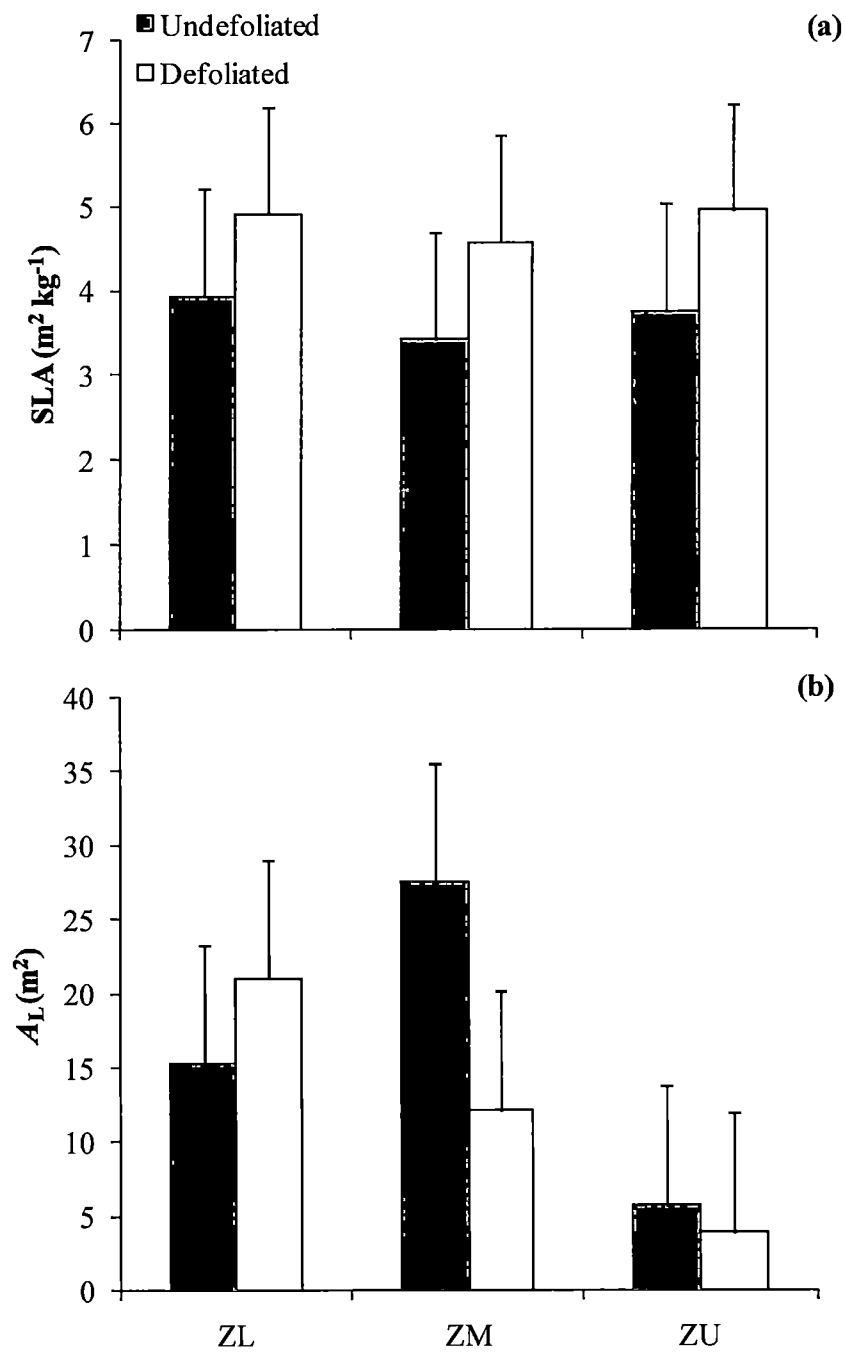


Figure 4.2. Defoliation treatment x crown zones interaction effects on (a) specific leaf area (SLA) and (b) leaf area (A_L) of undeveloped and defoliated *E. globulus* trees 322 days after treatment imposed. Values are means of three replicates \pm 95% LSD bars.

4.3.3. Branch variables

By the end of the experiment, defoliation treatment did not affect branch diameter and length ($P>0.1$; Table 4.1). In Z_L and Z_M , there was a 33% and 14% increase in the number of dead branches in control trees, compared to a 25% and 35% increase in defoliated trees, respectively ($P>0.1$; Figure 4.3a). In Z_U , defoliated trees also produced 25% less live branches than the undefoliated trees ($P>0.1$; Figure 4.3b).

4.3.4. Biomass partitioning and allocation

By the end of the experiment, mid-winter 2007, defoliation treatment did not affect the whole tree biomass of neither above- nor below-ground organs ($P>0.1$; Figure 4.4a), nor proportional allocation of biomass between organs ($P>0.1$; Figure 4.4b). Within crown zones, defoliation only significantly affected the foliage biomass in defoliated crown zone (Z_M) of the defoliated trees ($P<0.1$; Table 4.2), whereas there was no significant effect on the other organs ($P>0.1$; Table 4.2). Approximately 88% of biomass was allocated above-ground with more than 60% in Z_L and 30% respectively in Z_M ($P>0.1$), and 12% below-ground (Table 4.2). Only less than 4% of above-ground biomass was allocated to Z_U (Table 4.2). As a result of the defoliation, foliage biomass of defoliated trees was allocated more into Z_L than Z_M ; whereas undefoliated trees allocated more into Z_M than in Z_L (Table 4.2). In Z_U , defoliation treatment led to reduced allocation of foliage biomass but increased allocation of branch biomass compared to undefoliated trees ($P>0.05$; Table 4.2.).

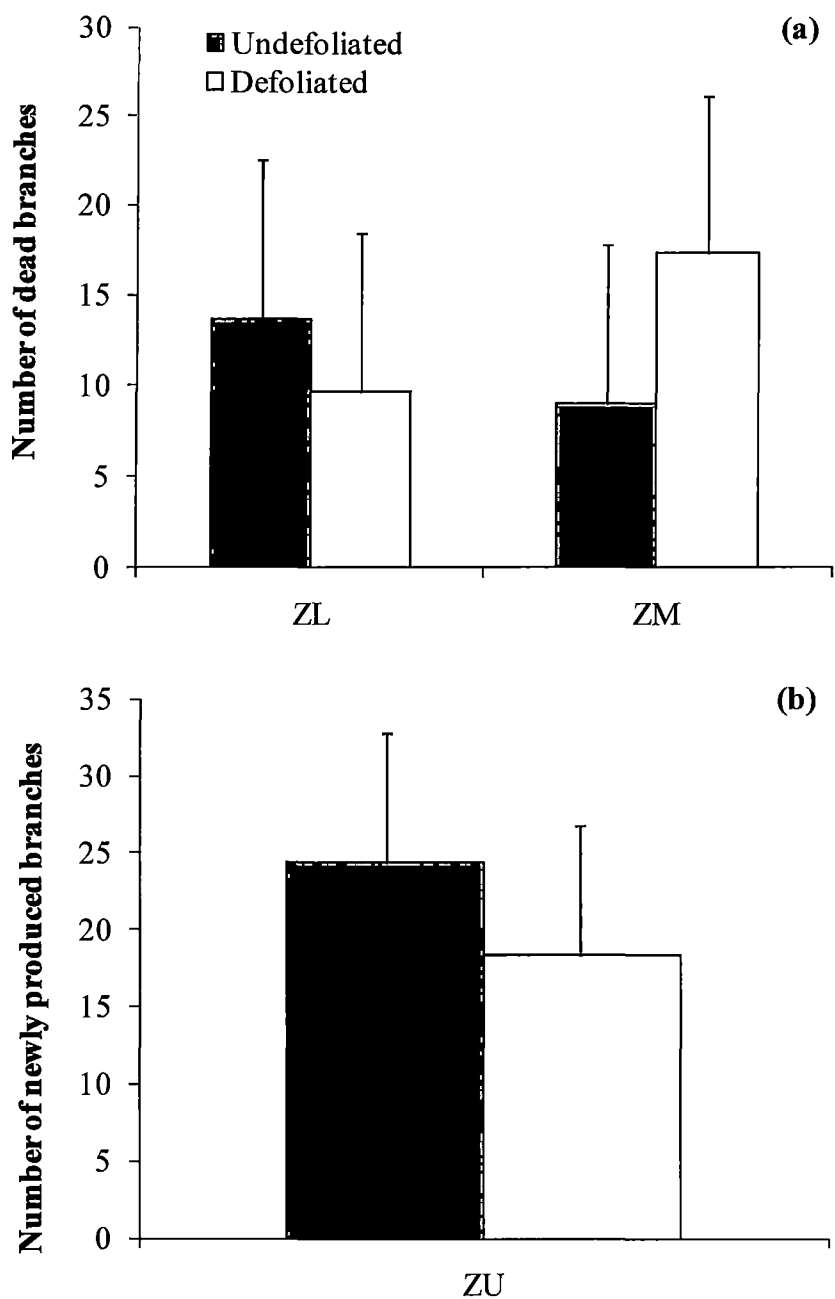


Figure 4.3. Defoliation treatment x crown zones interaction effects on the number of (a) dead branches in the lower (Z_L) and middle (Z_M) crown zones, and (b) newly produced branches in the upper crown zone (Z_U) of undefoliated and defoliated *E. globulus* 322 days after treatment imposed. Values are means of three replicates \pm 95% LSD bars.

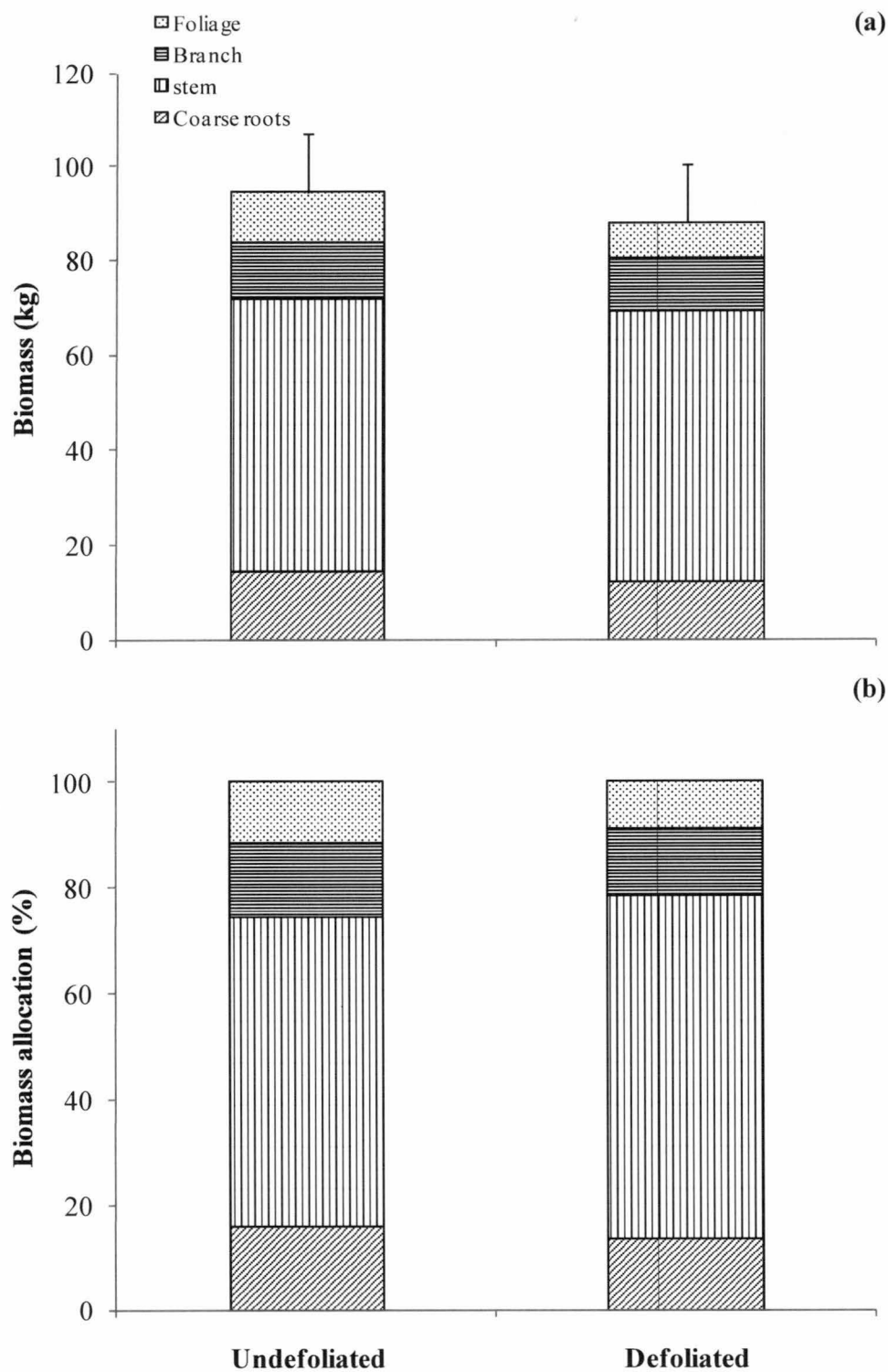


Figure 4.4. Mean biomass of whole-tree below-ground (coarse roots) and above-ground organs (stem, branch and foliage) of control and defoliated *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on 322 days after defoliation. Errors bars represent standard errors ($\alpha=0.05$).

Table 4.2. Variation of mean above-ground biomass (stem, branch, foliage and branch plus stem, also named wood), foliage to branch dry mass (FWR) and biomass allocation in the lower (Z_L), middle (Z_M), and upper (Z_U) crown zones of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation 322 days after treatment. Number in parentheses are least squares standard errors. Treatments having different letters indicate significant differences within crown zones or root class at $\alpha=0.05$ using LSD test.

Zones Treatments	Z_L		Z_M		Z_U	
	Undefoliated	Defoliated	Undefoliated	Defoliated	Undefoliated	Defoliated
<i>Biomass (kg tree⁻¹)</i>						
Stem	42 (13)	44 (7)	15 (7)	13 (2)	0.4 (0.2)	0.3 (0.1)
Branch	3.5 (0.6)	2.7 (1.2)	7.0 (0.6)	6.1 (1.0)	1.3 (0.6)	2.3 (1.4)
Foliage	2.9 (1.4)	4.1 (0.5)	6.6 (1.4)	2.6 (0.3) #	1.4 (0.9)	0.7 (0.2)
<i>Ratio</i>						
FWR	0.8 (0.4)	2.7 (1.5)	1.0 (0.2)	0.5 (0.5)	0.9 (0.3)	0.6 (0.0)
<i>Biomass allocation above-ground (%)</i>						
Stem [#]	52 (4)	58 (2)	16 (3)	17 (1)	0.4 (0.1)	0.4 (0.0)
Branch	5.3 (2.1)	3.8 (1.6)	10.2 (2.7)	8.0 (0.3)	1.4 (0.4)	2.7 (1.5)
Foliage	3.9 (2.2)	5.6 (0.9)	8.6 (1.1)	3.8 (0.9) #	1.4 (0.7)	1.0 (0.2)

4.3.5. Carbohydrate partitioning and dynamics

All above-ground organs showed higher soluble sugar than starch concentrations (Figure 4.5). Soluble sugar accounted for approximately 75% and starch for 25% of total non-structural carbohydrates. The highest soluble sugar and starch concentrations were found in leaves, then branches, then stems (Figure 4.5). Roots of defoliated and undefoliated trees displayed similar patterns for both soluble sugar and starch, with greater concentrations of starch than soluble sugar (Figure 4.5). Overall, defoliation treatment had no significant effect on branch, stem and coarse root carbohydrates concentration ($P>0.1$; Figure 4.5), whereas it significantly affected foliar soluble sugars and total non-structural carbohydrate concentration ($P<0.1$; Figure 4.5). Although there was no significant of the interaction between defoliation treatment and the crown zone or root classes ($P>0.1$), defoliation treatment markedly reduced foliar and branch soluble sugar within Z_U (Table 4.3). Additionally, a similar trend was observed in the soluble sugar of defoliated trees within 10 to 15 mm root diameter class (Table 4.3).

4.4. DISCUSSION

Tolerance to herbivore damage can be defined as a plant's capacity to compensate for lost leaf area and thereby maintain rates of growth at a level similar to those present pre-defoliation. In this study, four-year-old *E. globulus* trees were tolerant of partial defoliation applied as a single event. The evidence of the tolerance was that height and stem volume was unaffected, and this appeared to be achieved by reduced stem diameter growth, fewer branches and less foliage produced in Z_U , reallocation resources to maintain or support the production of foliage, and delay senescence of branches in the lower crown zone. Numerous studies have found that a range of tree species can compensate for partial defoliation (Bassman and Dickmann 1982; Pinkard and Beadle 1998b; Anttonen et al. 2002; Pinkard 2002; Alcorn et al. 2008; Eyles et al. 2009b).

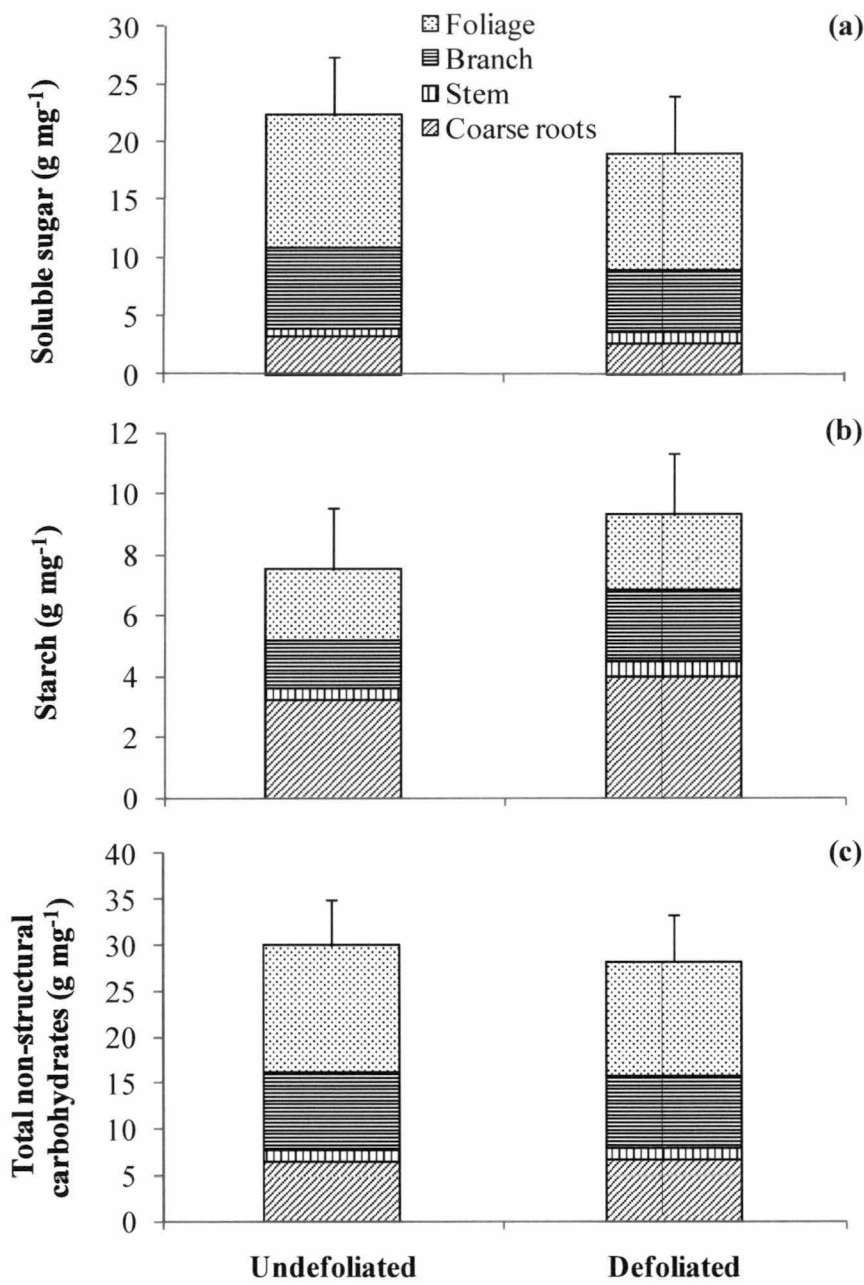


Figure 4.5. Mean concentration of (a) soluble sugar, (b) starch and (c) total non-structural carbohydrate of below-ground (coarse roots) and above-ground organs (stem, branch, foliage) of *E. globulus* trees growing in an experimental plantation in Southern Tasmania. Measurements were made on 322 days after defoliation.

Table 4.3. Mean soluble sugars, starch and total non-structural carbohydrates concentrations (g mg^{-1}) of each organ in the lower (Z_L), middle (Z_M), and upper (Z_U) crown zones of undefoliated and defoliated *E. globulus* trees growing in an experimental plantation in southern Tasmania measured at the harvest time (mid-winter). Treatments having different letters indicate significant differences within crown zones or root class at $\alpha=0.05$ using LSD test.

	Treatments	Soluble Sugar		Starch		TNC	
		Undefoliated	Defoliated	Undefoliated	Defoliated	Undefoliated	Defoliated
Foliage	Z_L	10.89 (± 0.76) a	11.1 (± 0.15) a	2.33 (± 0.48) a	2.08 (± 0.15) a	13.22 (± 0.30) a	13.17 (± 0.25) a
	Z_M	11.83 (± 0.25) a	9.83 (± 0.68) b	2.23 (± 0.18) a	2.40 (± 0.15) a	14.06 (± 0.22) a	12.23 (± 0.61) b
	Z_U	11.79 (± 0.47) a	9.01 (± 0.67) b	2.25 (± 0.17) a	2.96 (± 0.25) a	14.04 (± 0.62) a	11.97 (± 0.43) b
Branch	Z_L	5.5 (± 1.11) a	4.84 (± 0.57) a	1.57 (± 0.44) a	1.59 (± 0.52) a	7.06 (± 1.16) a	6.42 (± 1.02) a
	Z_M	5.9 (± 0.50) a	5.22 (± 1.23) a	1.96 (± 0.39) a	2.61 (± 0.49) a	7.86 (± 0.47) a	7.83 (± 1.60) a
	Z_U	9.44 (± 1.65) a	6.18 (± 0.72) b	1.19 (± 0.21) a	2.59 (± 0.36) a	10.63 (± 1.86) a	8.77 (± 0.87) a
Stem	Z_L	0.95 (± 0.32) a	1.32 (± 0.80) a	0.49 (± 0.04) a	0.55 (± 0.09) a	1.44 (± 0.31) a	1.87 (± 0.89) a
	Z_M	0.36 (± 0.14) a	0.64 (± 0.11) a	0.34 (± 0.15) a	0.47 (± 0.14) a	0.71 (± 0.29) a	1.11 (± 0.19) a
	Z_U	0.93 (± 0.18) a	0.89 (± 0.10) a	0.52 (± 0.14) a	0.44 (± 0.13) a	1.44 (± 0.31) a	1.33 (± 0.23) a
Roots	C_{5-10}	3.69 (± 0.72) a	3.30 (± 0.40) a	3.87 (± 1.49) a	3.89 (± 2.66) a	7.57 (± 2.14) a	7.19 (± 2.50) a
	C_{10-15}	3.92 (± 0.27) a	2.28 (± 0.39) a	2.01 (± 0.51) a	3.62 (± 0.31) a	5.93 (± 0.78) a	5.91 (± 0.68) a
	C_{15-20}	4.03 (± 1.54) a	3.17 (± 0.41) a	3.10 (± 2.15) a	4.54 (± 1.56) a	7.13 (± 3.66) a	7.71 (± 1.68) a
	$C_{>20}$	2.64 (± 0.47) a	2.81 (± 0.87) a	4.05 (± 1.40) a	5.51 (± 2.25) a	6.69 (± 1.60) a	8.33 (± 3.12) a
	Rootball	1.68 (± 0.19) a	1.23 (± 0.27) a	3.07 (± 1.59) a	2.54 (± 0.61) a	4.75 (± 1.57) a	3.78 (± 0.65) a

This includes *E. globulus* although all studies to date on this species have focused on young trees prior to canopy closure (Pinkard 2003; Pinkard et al. 2006b; Pinkard et al. 2007; Eyles et al. 2009b).

In this study of *E. globulus* trees post canopy closure, only short-term effects on diameter growth were observed ($P < 0.05$; Figure 4.1); there was no effect on height or volume growth. The link between plant growth and resource availability is well established (Jarvis and Leverenz 1983). Irrigation and added fertiliser ensured that the demand for water and nutrients following defoliation should not have been a factor limiting the growth response. Hence the observed differences between the treatments are more likely related to defoliation temporarily reducing photosynthetic performance of crowns, in spite of compensatory responses. It has been observed previously that pruning of *E. nitens* at canopy closure (Pinkard and Beadle 1998b) and of *E. globulus* at pre-canopy closure (Pinkard 2003) has less effect on growth at more productive than less productive sites and that diameter growth is affected to a greater extent than height growth. In the current experiment, the lack of significance at the entrance of winter may suggest that defoliated *E. globulus* trees have been capable of maintaining growth rate of diameter, while that of control trees was slightly slowing down (see Figure 4.1). Additionally, there was an indication from this study that defoliation may negatively impact stem wood density in a short term, though this difference was not significant, and therefore any potential consequence on timber quality remains uncertain. Lower wood density in pruned *E. grandis* seedlings has been associated with thinner fibre cell walls and this has been attributed to reduced supply of carbohydrates to the wood fibre cells, resulting in thinner cell walls (Thomas et al. 2006).

One strategy that can mitigate the negative effects of herbivore attack is the alteration of carbon allocation patterns and the mobilisation of stored reserves to accelerate the replacement of damaged tissue (Dickson 1989; Reich et al. 1993; Trumble et al. 1993; Vanderklein and Reich 1999). In this experiment, defoliated trees maintained similar levels of biomass in all plant organs to control trees; total biomass increment was also not adversely affected by defoliation. Thus defoliated trees appeared to compensate quite rapidly to the treatment. Previous experiments using partial (50-66%) defoliation in other tree species have reported significant reductions in total above-ground biomass

production (Reich et al. 1993; Krause and Raffa 1996) or no reduction (Ericsson et al. 1980; Vanderklein and Reich 1999). Such contrasting results once again point to the productive potential of the site determining the rate of recovery from defoliation (Maschinski and Whitham 1989). In *E. globulus*, studies on the interactive effects of defoliation and abiotic factors have shown that adequate resource availability often favours the expression of tolerance (Pinkard et al. 2006b; Pinkard et al. 2007), though Eyles et al. (2009b) was unable to discriminate tolerance of five-month-old saplings that received contrasting levels of water and nutrient supply as compensatory responses were attributed to the activation of a number of short and longer-term physiological mechanisms. In the Pinkard et al. (2007) study, recovery responses were linked to the application of nitrogen before the defoliation event.

Defoliation had little influence on the concentration of carbohydrates in branch, stem and root. There was also very little evidence of reduction of starch in roots or stems. This may suggest that carbohydrates were either not utilised amongst these tree components. A significant decrease of foliar soluble sugar concentrations in Z_M and Z_U zones in defoliated trees indicates the depletion of current assimilates for the production of new leaves. In five-month-old saplings of *E. globulus*, defoliation resulted in a lower concentration of foliar and root-stored carbohydrates, suggesting that very young trees are more reliant on carbohydrate reserves to overcome the negative carbon balance imposed by defoliation (Eyles et al. 2009b).

Defoliation of in the Z_M zone elicited replacement of leaf area in this part of the crown, but reduced the production of new branches and leaves in the Z_U zone. The higher rates of leaf area growth of defoliated trees were accompanied by an increase in specific leaf area. Growth following defoliation typically produces leaves of high SLA (Oosterheld and McNaughton 1991) and indicates a more efficient use of resources for light capture (Hodgkinson 1974; Alderfer and Eagles 1976; Trumble et al. 1993). Defoliation also induced substantial branch death in Z_M compared to that observed in the controls; conversely branch death in Z_L in control was greater than that in defoliated trees. This latter observation was possibly related to greater light penetration into the lower canopy following defoliation (Nowak and Cadwell 1984). In addition, defoliation in eucalypt (Pinkard and Beadle 1998b) and other trees species (Nowak and

Cadwell 1984; Trumble et al. 1993) delays the onset of branch and leaf senescence that is possibly related to a shift in hormonal balance between shoot and roots (Trumble et al. 1993). The senescence of branches within Z_M of defoliated trees was mostly observed in the lower part of Z_M . Additionally, the loss of foliage in this lower part has not been replaced while intense refoliation occurred in the upper part of Z_M . It likely that the absence of refoliation, which subsequently generated the deficiency in assimilate supply necessary for the support of the crown architecture, caused the senescence of the branches.

In spite of increased branch death, rates of leaf area production in Z_M (upper part) were higher than in control trees, an observation that may also be related to compensatory photosynthesis following defoliation. Refoliation of this defoliated crown zone was clearly a greater priority for carbon utilisation than the vertical development of new foliage in Z_U . In the previous pot experiment (see Chapter 3), selective defoliation of young *E. globulus* seedlings was observed to increase the sink strength of the damaged branches. Source-sink relationships (Haukioja et al. 1990; Honkanen and Haukioja 1994; Honkanen et al. 1994; Honkanen et al. 1999) mean that the removal of upper crown foliage eliminates immediate sources of carbohydrate for existing apical growth; at the same time, pre-existing sources are more readily accessible. In this study, the assimilates in undamaged Z_L are potentially available to meet demand from pre-existing sinks (in Z_M), particularly as there is a greater energy cost to the plant in transporting carbohydrates to new actively growing zones like Z_U (Küppers 1989). This may explain the lower ratio of branch leaf area to branch cross-sectional area in Z_U indicating that the supply of carbon for new growth was limited.

Overall, defoliated mature trees were able to tolerate 45%-defoliation of the upper crown. Interestingly, responses of mature *E. globulus* were similar to the response observed with younger *E. globulus*. Our results partially supported the first hypothesis that defoliated *E. globulus* maintained stem growth at the expense of other biomass pools. In the short term, partial defoliation reduced the diameter growth of *E. globulus*, but did not affect biomass allocation. Changes in crown architecture were suggested by the results, indicating that the loss of leaf area resulted in accelerated rates of refoliation within the defoliated crown zone, and moderately expanded to the new

crown which partly valid the second hypothesis. There was also strong support for the third hypothesis that current carbohydrates were used to replace foliage lost to maintain growth. Results in other studies suggest that a minimum level of leaves and branches is required for the maintenance of stem growth (Pinkard and Beadle 1998a; 1998b). Below this level, assimilates are diverted to support and increase the development of leaves and branches. In *E. nitens*, 70%-pruning reduced the proportion of leaves and branches and did not allow sustaining growth (Pinkard and Beadle 1998a; 1998b). It is highly likely that similar results would be obtained with *E. globulus* in closed canopy. However, further research, covering a range of defoliation severities, is required.

A single defoliation event removing the upper half of crown will likely have little effect on mature *E. globulus* when resources are not limited, and similar responses were observed with younger *E. globulus*. However, defoliation may have more effect where resources are limited. Indeed, studies have suggested that eucalypts growing on more fertile sites are better able to recover from leaf removal than those experiencing some level of stress (Pinkard 2003; Eyles et al. 2009b). The site factor that can be most easily manipulated silviculturally is nutrient availability. There is ample evidence that eucalypts can respond to fertiliser applications, particularly of nitrogen by increasing leaf area and hence stem growth (Smethurst et al. 2003). In such circumstances fertilising with nitrogen may promote crown recovery and may be a potential management tool for dealing with defoliation events (Pinkard et al. 2006b). Assessing tree response to simulated herbivory in various growing conditions may help the development of sophisticated tools for pest management. However, artificial defoliation studies do not necessarily mimic what occurs in the real world (see Chapter 3) but they can provide a sounder basis for understanding the effects of insect defoliation that can then be used to develop management strategies. Because it is very difficult to control levels of defoliation or infection from foliar pathogens in the field, artificial defoliation studies especially on smaller trees are useful in the examination of growth responses to defoliation. Yet, studies covering a range of defoliation severities and abiotic treatments using both artificial and insects should be the focus of further research.

Chapter 5. Traits regulating photosynthesis.

5.1. INTRODUCTION

Increased leaf-level photosynthetic activity following defoliation by herbivores is a trait that has long been considered indicative of plant tolerance to herbivory (McNaughton 1983; Strauss and Agrawal 1999) and associated with plant defence (Karban and Baldwin 1997). Such up-regulation of photosynthesis or photosynthetic compensation (Welter 1989) may promote tree recovery from herbivory by increasing the photosynthetic capacity of remaining leaves (Reich et al. 1993; Kolb et al. 1999; Vanderklein and Reich 1999). The ability of plants to increase their carbon-fixing capacity (Field 1983) has often been identified as an expression of photosynthetic compensatory response.

Large amount of the nitrogen within leaves is found in photosynthetic enzymes (Evans 1989) and a strong correlation is often observed between photosynthetic capacity and leaf nitrogen concentration (Leuning et al. 1991; Sheriff and Nambiar 1991). Removal of active leaf area during herbivory inevitably reduces the pool of available photosynthate (Reich et al. 1993; Kolb et al. 1999; Vanderklein and Reich 1999). Typically, starch concentrations in remaining foliage are reduced following partial leaf removal (Ericsson et al. 1980). However, the mechanisms by which up-regulation of photosynthesis occurs to offset these changes are poorly understood. Some studies have concluded that an increase in photosynthetic rate is mechanistically linked to increases in foliar nitrogen concentrations (Hoogesteger and Karlsson 1992); others have found no such relationship (Lovett and Tobiessen 1993; Ovaska et al. 1993b; Reich et al. 1993). Increases in photosynthetic rate have also been linked to changes in stomatal or intercellular conductance (Heichel and Turner 1983) or rate of carboxylation (Ovaska et al. 1993b). Alternatively, defoliation leads to increased photosynthesis as a result of decreased starch formation which normally limits photosynthesis in undefoliated plants (Foyer 1987).

Most plants compensate for defoliation to some degree, but the level of compensation varies widely between species and even individuals of the same species (Trumble et al. 1993). Plant responses to partial defoliation have mainly been examined using small plants or seedlings; studies using trees are rare but those undertaken show that the extent to which physiological and morphological responses are expressed vary considerably (Trumble et al. 1993; Vanderklein and Reich 1999; Vanderklein and Reich 2000). External factors such as the degree of canopy closure and environmental conditions (Pinkard et al. 2007), the amount of foliage removed (Hoogesteger and Karlsson 1992; Reich et al. 1993; Pinkard et al. 1998), the timing (Pinkard et al. 2007), the pattern (Elek 1997; Collett and Neumann 2002) and the frequency (Wills et al. 2004; Pinkard et al. 2007) of defoliation contribute to this variety of responses.

In this study, I investigated physiological responses to upper crown defoliation caused by artificial defoliation of four-year old plantation *E. globulus* trees post canopy closure and under non-limiting water and nutrient supply. Experimentation to obtain data that can be used to predict the level of growth impact caused by herbivore damage to trees is logistically difficult. Artificial defoliation was used because natural herbivory cannot be predicted or controlled in the field. The small size of the sample inevitably influenced the power of the statistical analysis and therefore affected the statistical significance of measured differences between treatments. Because of this some reliance is placed on marked differences in biological trends to make statements about treatment effects. In this study, the hypotheses tested were:

1. photosynthetic up-regulation occurs in the remaining lower crown zone;
2. newly developing foliage in the upper crown is also up-regulated as part of the response to defoliation;
3. defoliation increased foliar nitrogen content;
4. stored and new carbon sources are mobilised to accelerate the development of the new upper crown zone;
5. photosynthetic up-regulation is explained by changes in stomatal conductance and not in foliar nitrogen content

5.2. MATERIALS AND METHODS

5.2.1. Treatments

The experiment was carried out in a single plot irrigated with 500 mm municipal water per annum. Irrigation was applied every second night and, with incident rainfall, ensured a relatively uniform supply of soil water throughout the soil profile. Soil moisture was monitored monthly at the site using a neutron moisture meter (NMM, Hydroprobe, CPN503, CA, USA). Granulated fertiliser was applied evenly across the site at three-monthly intervals. The composition and concentration of macro- and micronutrients are given in O'Grady et al. (2005). Weed control, both manual and chemical with glyphosate was used to maintain the site weed free.

The experiment was undertaken over a period of 217 days between late winter 2006 (August) and autumn 2007 (April). Six trees of 13.0 to 13.2 m height and with diameter at 1.3 m between 115 and 168 mm were selected at the start of the measurement period. An elevated work platform was used to access the tree crowns for measurements.

The trees used in this experiment were the same trees as in Chapter 4. Prior to defoliation, the crowns of all trees were divided into two vertical (upper, lower) crown zones; the lower crown was then divided into two horizontal (inner, outer) crown zones. Figure 5.1 illustrates the division of the crown. At the start of the experiment, upper- and lower-crown zones were delimited by half crown length. At each measurement time, inner- and outer-crown in the lower crown zone were delineated by half crown width taking into account any branch extension. Three trees were selected randomly for the defoliation treatment, and three were selected as controls (undefoliated). The defoliation treatment was applied late winter (29-30/08/06). All leaves except the last expanding leaf pair from the end of the previous growing season were removed from all first and lower order branches by snipping the petiole flush with the branch from the upper zone to approximately half crown height. We removed 45% of total leaf area, as estimated from the allometric relationships of O'Grady et al. (2006).

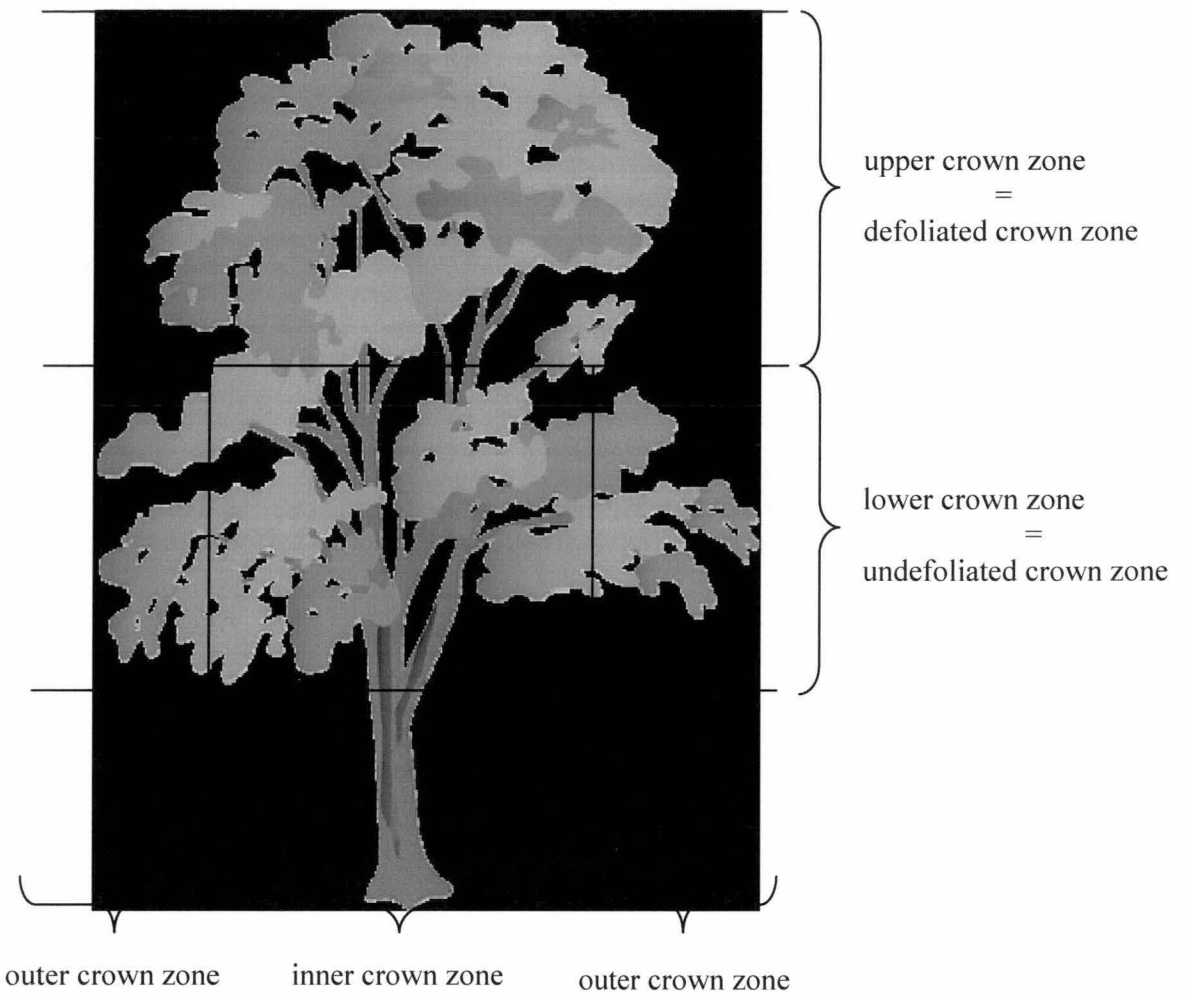


Figure 5.1. Illustration representing the delineation of the three crown-zone prior to defoliation.

5.2.2. Gas exchange

Gas exchange and leaf traits were measured on the six trees and in each crown zone. Maximum light-saturated CO₂ uptake (A_{\max}), and leaf stomatal conductance (g_s) were measured with an open flow infra-red gas analyser (CIRAS-1, PP Systems, Herts, UK). The leaves were enclosed in a 250 mm² leaf chamber fitted with a light source providing a photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The ambient CO₂ concentration was maintained at 360 ppm. Leaf temperature varied between 16 and 21°C, and the readings were made when g_s had stabilised. A_{\max} and g_s were measured on five occasions after the application of the defoliation: late winter (31/08/06), mid-spring (06/10/06), early summer (14/12/06), mid-summer (22/01/07), and early autumn (26/03/07).

In each tree and each crown zone, measurements were made on four healthy fully-expanded leaves between 1000 and 1400 h AEST. Leaves selected in the lower crown zones were fully-expanded leaves from the previous season. Leaves selected in the upper crown zone in spring were from the leaf pair retained after defoliation as newly produced leaves were still expanding (Beadle and Turnbull 1986). This was done to ensure that measurements of photosynthesis and chemical extraction were done on mature leaves. However as this last leaf pair had emerged at the end of the previous growing season, these were not fully expanded though had ceased growing. For later measurements, leaves from the upper crown zone had all developed after the defoliation and were fully expanded. Leaves in the non-defoliated lower crown zones were all fully expanded and collected from mid-canopy (inner) and at the extremity of the canopy (outer).

5.2.3. Specific leaf area and chemical analyses

After completion of each set of gas exchange measurements, the selected leaves were excised and stored on ice for a maximum of 3 h until removed to the laboratory for analysis of foliar chemistry and specific leaf area (SLA). The leaves were dried at 65°C for 48 h to constant mass and finely ground in a hammer mill.

Following the method of Lowther (1980), 10 mg samples were prepared for nitrogen (N) and phosphorus (P) analyses using the single acid-hydrogen peroxide technique. Total nitrogen and phosphorus concentrations were measured using a continuous flow colorimetric autoanalyser (McLeod 1992). Non-structural carbohydrates were extracted from 50 mg samples in 10 mL of 80% (v/v) ethanol in a 60°C water bath (Palacio et al. 2007). Carbohydrates remaining in the undissolved pellet after ethanol extraction were digested to glucose with amyloglucosidase from *Aspergillus niger* (Fluka-10115; BioChemika, Switzerland). The concentrations of soluble sugar and starch were determined using a phenol/sulphuric acid colorimetric assay (Dubois et al. 1956; Buysse and Merckx 1993). Non-structural carbohydrates measured after the ethanol extraction are referred to as soluble sugars, carbohydrates measured after the enzymatic digestion are referred to as starch, and the sum of soluble sugars and starch measured in glucose equivalents are referred to as non-structural carbohydrates. Concentrations were initially calculated on a dry mass basis and then converted to an area basis using SLA.

5.2.4. Data analysis

A repeated measure ANOVA was used to explore how the defoliation treatment effects develop on A_{\max} , g_s , SLA, nitrogen, phosphorus, soluble sugar, starch, non-structural carbohydrates, and nitrogen-use efficiency (NUE) within crown zone. The repeated measures ANOVA test the equality of means. In cases where there is a great deal of variation between sample members, error variance estimates from standard ANOVAs are large. Repeated measures of each sample member provide a way of accounting for this variance, thus reducing error variance. Analyses were carried out using Genstat10 software (GENSTAT Committee 1989).

A group regression procedure was used to explore the relationship between A_{\max} and g_s , and foliar nitrogen concentration with defoliation treatments (undefoliated and defoliated) as groups (McPherson 1990). This method provides estimated regression equations under different models where groups are present in the data. This procedure tests the hypotheses that (1) the regression lines have common slope allowing for the

possibility that they have different intercepts, and (2) that the same relationship applies to both defoliation treatments. Data were log transformed to restore normality. All regressions were performed using Genstat10 software.

5.3. RESULTS

5.3.1. Specific leaf area

The overall mean specific leaf area (SLA) of defoliated trees ($4.92 \pm 0.1 \text{ m}^2 \text{ kg}^{-1}$) was significantly higher than that of undefoliated trees ($4.52 \pm 0.1 \text{ m}^2 \text{ kg}^{-1}$) treatment over the measurement period ($P < 0.1$). Mean SLA showed a marked change within the upper crown zone of defoliated trees between spring (Oct 06) and early summer (Dec 06) (Table 5.1) that was probably linked to the switch from sampling leaves that had developed pre-defoliation to those developed post-defoliation. Mean SLA of undefoliated and defoliated trees was relatively constant with time, irrespectively of crown zone ($P > 0.1$). Crown zone had a highly significant effect on SLA throughout the measurement period ($P < 0.001$). Mean SLA of undefoliated trees decreased with increasing crown height ($P < 0.1$). In contrast, mean SLA of defoliated trees was similar between crown zones ($P > 0.1$; excluding measurement in spring). In the upper crown zone, the defoliation treatment resulted in higher SLA than the control mid-summer (Jan 07), 146 days after defoliation ($P < 0.1$; Table 5.1). In the lower outer crown zone, a significant difference between the two treatments was visible only in spring (Oct 06) with higher SLA in the defoliated than the undefoliated trees ($P < 0.1$; Table 5.1). In the lower inner crown zone, there were no significant differences between treatments at any measurement time ($P > 0.1$; Table 5.1).

Table 5.1. Repeated measure ANOVA for effects of defoliation treatments on whole tree physiological response. Values are means of three replicates with SE in parentheses.

Variables	Undefoliated	Defoliated	P-value
Specific leaf area (SLA; $\text{m}^2 \text{kg}^{-1}$)	4.525	4.908	0.049
Maximum photosynthesis (A_{max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	10.35	13.46	0.027
Stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1}$)	180.3	243.6	0.016
Foliar nitrogen content (g m^{-2})	2.464	2.322	0.602
Foliar phosphorus content (g m^{-2})	0.224	0.221	0.915
Nitrogen use efficiency (NUE; $\mu\text{mol g}^{-1} \text{s}^{-1}$)	5.30	6.24	0.133
Foliar soluble sugar content (g m^{-2})	19.65	19.84	0.873
Foliar starch content (g m^{-2})	4.90	3.97	0.152
Foliar total non-structural carbohydrates content(TNC g m^{-2})	24.54	23.22	0.421

Table 5.2. Mean specific leaf area (SLA) of undefoliated and defoliated trees in lower inner, lower outer and upper crown zones from spring (Oct 06) to autumn (Mar 07). Standard errors are in parentheses. *indicates significant differences between control and defoliation treatments at $P < 0.1$.

Date	Crown zones	SLA ($\text{m}^2 \text{kg}^{-1}$)	
		Undefoliated	Defoliated
Oct 06	Lower inner	4.79 (0.10)	5.32 (0.17)
	Lower outer	4.80 (0.44)	5.66 (0.31)*
	Upper	4.39 (0.20)	4.05 (0.15)
Dec 06	Lower inner	4.44 (0.09)	4.71 (0.36)
	Lower outer	4.51 (0.28)	4.71 (0.24)
	Upper	4.39 (0.19)	5.08 (0.02)*
Jan 07	Lower inner	4.81 (0.21)	4.94 (0.43)
	Lower outer	4.79 (0.34)	5.44 (0.35)
	Upper	4.16 (0.24)	5.06 (0.1)*
Mar 07	Lower inner	4.51 (0.15)	4.37 (0.24)
	Lower outer	4.48 (0.36)	4.75 (0.26)
	Upper	4.25 (0.23)	4.97 (0.26)*

5.3.2. Light-saturated photosynthetic rate and stomatal conductance

Throughout the study, A_{\max} and g_s were significantly higher in defoliated trees than the undefoliated trees ($P < 0.1$; Table 5.1). The effect of crown zone was also highly significant on A_{\max} and g_s ($P < 0.1$). The smallest A_{\max} and g_s values were measured in foliage of lower crown zones and the greatest were always in the foliage of the upper crown zone (Figure 5.2, 5.3); A_{\max} and g_s also differed significantly between inner and outer lower-crown zones, and was always lower in the inner compared to the outer zone ($P > 0.1$; Figure 5.2, 5.3).

In the upper crown zone, A_{\max} was significantly higher in the defoliated than undefoliated trees in early summer (Dec 06) only ($P < 0.1$; Figure 5.2a); g_s was significantly higher in the defoliated than undefoliated trees from spring (Oct 06) to mid-summer (Jan 07) ($P < 0.1$; Figure 5.3a). In the lower outer zone, A_{\max} was significantly higher in the defoliated than undefoliated trees from spring (Oct 06) to mid-summer (Jan 07) ($P < 0.1$; Figure 5.2b); g_s was significantly higher in the defoliated than undefoliated trees during summer ($P < 0.1$; Figure 5.3b). In the lower inner crown zone, A_{\max} and g_s were significantly higher in the defoliated than undefoliated trees only in Jan 07 ($P < 0.1$; Figure 5.2c, 5.3c). The highest percentage increase in A_{\max} following defoliation occurred mid-summer (Jan 07) in the foliage of the lower crown zone, where A_{\max} of defoliated trees was up to 59% higher than that of undefoliated trees (Figure 5.2).

5.3.3. Relationship between light-saturated photosynthetic rate and stomatal conductance

For all measuring day, the relationship between A_{\max} and g_s was linear and highly significant in both treatments ($P < 0.001$). However, defoliation treatment changed significantly the relationship in spring 2006 ($P < 0.1$; Figure 5.4). When data from the three crown zones were pooled, defoliation treatment significantly decreased the intercept of the relationships in comparison to the undefoliated treatment ($P < 0.05$; Figure 5.4).

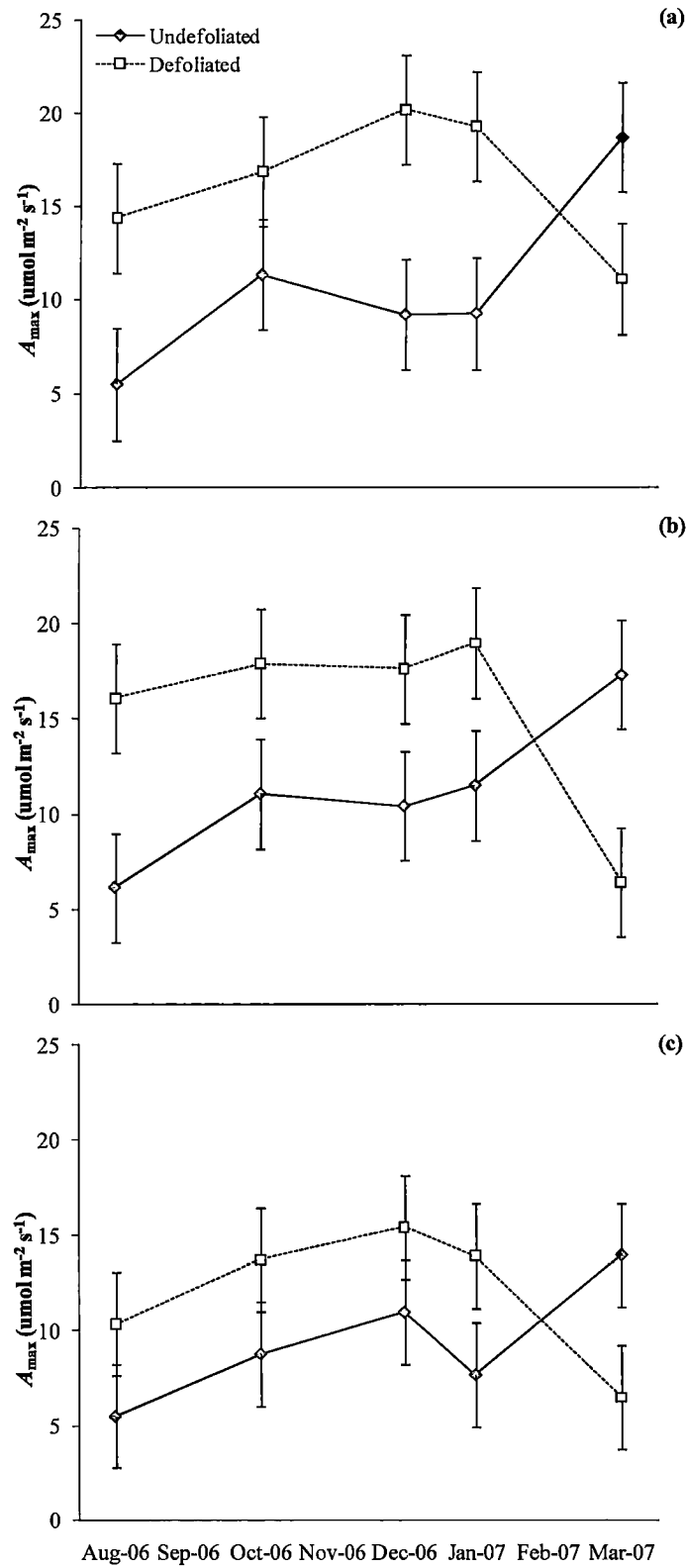


Figure 5.2. Mean light-saturated photosynthetic rates (A_{max}) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliated treatments at Pittwater from late winter 2006 to autumn 2007. Error bars show mean standard errors ($\alpha=0.05$). Homogeneous groupings are identified by letters ($\alpha=0.05$).

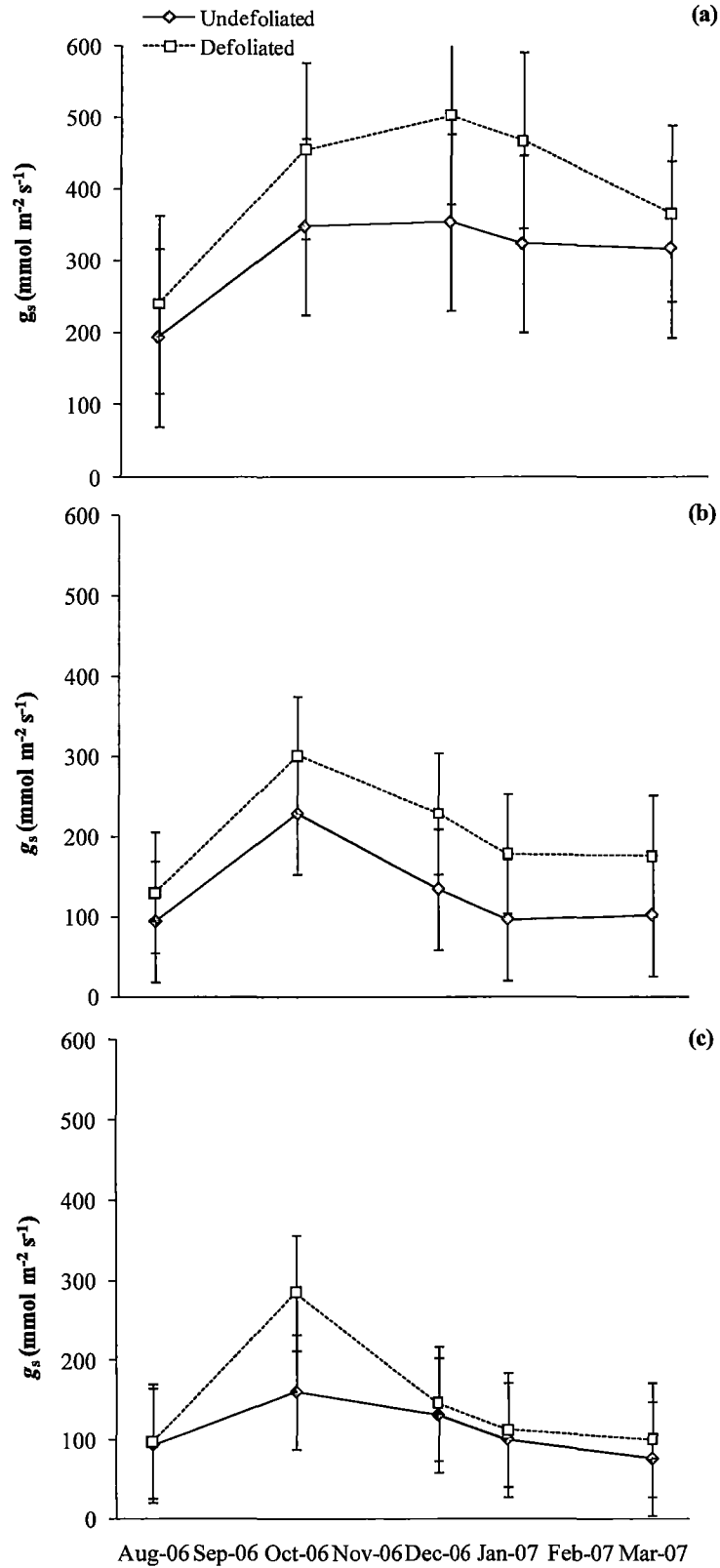


Figure 5.3. Mean leaf stomatal conductance (g_s) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliated treatments at Pittwater from late winter 2006 to autumn 2007. Error bars show mean standard errors ($\alpha=0.05$). Homogeneous groupings are identified by letters ($\alpha=0.05$).

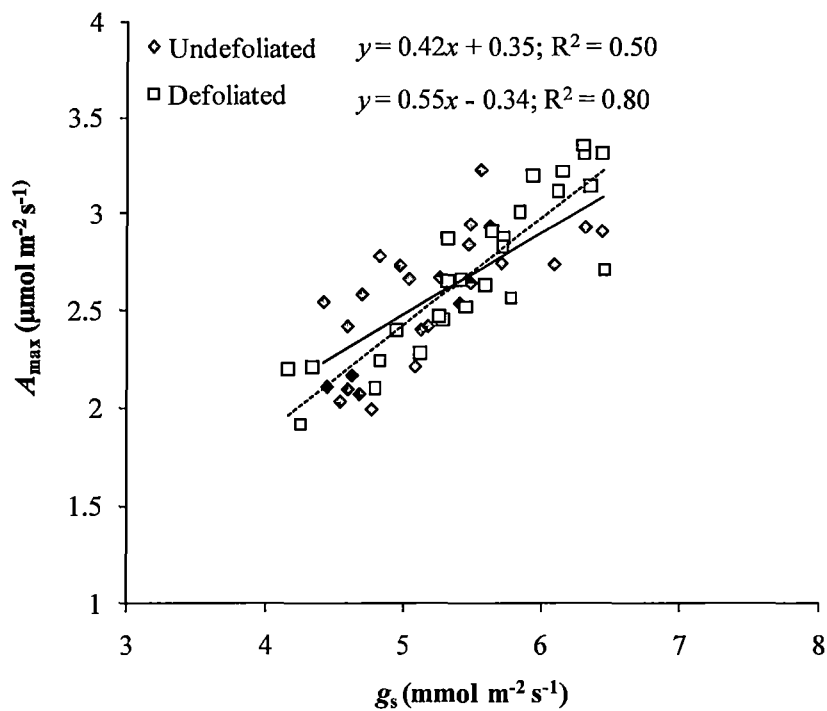


Figure 5.4. Log-log transformed relationship between light-saturated photosynthetic rate (A_{\max}) and stomatal conductance (g_s) of upper (a), lower outer (b) and lower inner (c) crown zones of trees in undefoliated and defoliation treatments in an experimental plantation in Southern Tasmania. Trendlines are represented for pooled data in spring 2006 for undefoliated (solid line) and defoliated (dotted line) *E. globulus* trees.

5.3.4. Foliar nitrogen, phosphorus and carbohydrate contents

Area-based foliar nitrogen concentration varied by crown zone throughout the measurement period ($P < 0.01$) and generally increased with crown height for both undefoliated and defoliated treatments. However, nitrogen of defoliated trees did not differ significantly to nitrogen of the undefoliated trees ($P > 0.1$; Table 5.1). Positive correlations between foliar nitrogen content and A_{\max} were found in both treatments ($P < 0.001$). Defoliation treatment had no significant influence on the relationship ($P > 0.1$). Defoliation treatment or crown zone had no effect on area-based phosphorus concentrations throughout the study period ($P > 0.1$; Table 5.1). There was no evidence of relationship between foliar phosphorus content and A_{\max} . Foliar carbohydrate concentrations were less in the defoliated than undefoliated trees ($P > 0.1$; Table 5.1). There was no evidence of relationship between foliar carbohydrates content and A_{\max} .

5.3.5. Nitrogen use efficiency

In each treatment, photosynthetic nitrogen-use efficiency (NUE) was less at the end than beginning of the experiment. Crown zone did not have a significant effect on NUE ($P > 0.1$); the highest NUE values were generally found in the upper crown. There was no evidence of a significant difference in NUE between undefoliated and defoliated treatments ($P > 0.1$; Table 5.1).

5.4. DISCUSSION

This study has clearly demonstrated that maximum photosynthesis and stomatal conductance of *E. globulus* leaves increases following defoliation in the upper crown of 4-year-old trees (Figures 5.2 and 5.3). This photosynthetic enhancement or up-regulation that occurred throughout the canopy has been shown to play an important role in moderating the impact of partial defoliation on growth. For example, enhanced

photosynthesis following 50%-pruning in *E. nitens* increased net biomass production to a similar level as unpruned trees (Pinkard and Beadle 1998a; Pinkard et al. 1998). Reich et al. (1993) reported that removal of up to 50% of leaf area of *Pinus resinosa* had no impact on growth of seedlings 14 months later, which they attributed to enhanced photosynthetic rates and changes in patterns of carbon allocation to favour leaf development. In the current experiment, 45%-defoliation had no significant impact on tree growth 11 months later, and refoliation of the upper crown zone was a high priority for carbon utilisation (see Chapter 4).

In contrast with studies on seedlings and saplings (e.g. Lovett and Tobiessen 1993; Ovaska et al. 1993b), including *E. globulus* (Turnbull et al. 2007; see chapter 3) and *E. nitens* (Pinkard et al. 1998) where significant increases in A_{\max} occurred within two weeks of defoliation, the up-regulation of A_{\max} of the remaining leaves of 4-year-old *E. globulus* in this experiment was not observed until 5 weeks (Oct 06) after defoliation; however this occurred only in the lower outer canopy, possibly because the leaves in the upper canopy were not fully expanded. Newly-formed leaves can have insufficient photosynthetic capacity to sustain any demand for carbon (see Heichel and Turner 1983) and are therefore unlikely to up-regulate, although in this experiment these leaves had measured A_{\max} similar to those observed in the upper canopy later in the season. Because leaves of temperate eucalypts require about 10-12 weeks to fully expand after their emergence from apical buds (Beadle and Turnbull 1986), no up-regulation in A_{\max} was observed in the upper canopy of defoliated *E. globulus* until 15 weeks after defoliation (Dec 06). Up-regulation of leaves in the lower inner zone was only observed once, 20 weeks after defoliation, a finding that may relate to these being the oldest leaves sampled. Trees have a larger capacity for carbon storage than seedlings or saplings for use in times of great demand such as following a defoliation event (Cannell and Dewar 1994). The delay in the onset of photosynthetic up-regulation observed following defoliation in this study suggests that the trees were able to buffer reductions in carbohydrate production following defoliation by drawing on such reserves of stored carbon.

Up-regulation of photosynthesis was sustained for a maximum of 15 weeks in the upper crown zone, and 20 weeks in the lower outer crown zone. Studies using partial

leaf removal report increased photosynthetic rates in *Acer rubrum* L. and *Quercus rubra* L. (Heichel and Turner 1983) and *P. resinosa* (Reich et al. 1993) being sustained for a duration of about 6 months. In contrast, photosynthetic up-regulation in 3-year-old *E. nitens* was measured for up to 16 months following green pruning (Pinkard et al. 1998). Green pruning removes branches from the base of the live crown upwards, inducing a condition known as “source limitation” (Pinkard and Beadle 1998c). Defoliation of trees by insects, as was mimicked in this experiment, often occurs on young foliage in the upper part of the canopy, potentially inducing a “sink limitation” (Baysdorfer and Bassham 1985). The use of such a sink/source hypothesis about how leaves compete for resources provides a framework for understanding up-regulation of photosynthesis (Honkanen and Haukioja 1994). In this experiment, the up-regulation of A_{\max} in the lower outer crown following defoliation occurs in response to a growing demand for assimilates to support replacement of the upper crown leaf mass. The short duration of the photosynthetic response of defoliated *E. globulus* may have reflected the time taken to increase leaf area to pre-defoliation level. In pruned trees, the carbon fixed and stored in the foliage and stored in branches of the lower crown zone is no longer available, therefore the up-regulation of photosynthesis in the residual crown has to be prolonged over a longer period of time to meet the demand for carbon.

Although the increases in A_{\max} observed after defoliation were mainly associated with significant up-regulation of photosynthesis in the lower outer crown zone, the younger foliage in the upper crown zone had greater A_{\max} than older foliage throughout the experiment. Maximum rates of photosynthesis are a function of rates of biochemical reactions that are determined by the intracellular resistance to CO_2 transfer of the leaf and levels of incident light (Farquhar et al. 1980). Although not measured, defoliation of the upper canopy might have increased average levels of incident light in the lower canopy, increasing intracellular conductance and therefore A_{\max} , and possibly reinforcing the up-regulation of photosynthesis (Anten and Ackerly 2002). However it should be noted that seasonal increases in A_{\max} of control trees were also observed concurrently during the experiment.

Changes in specific leaf area can be used to interpret the distribution of incident light through the canopy (James and Bell 2000). As in other studies (Oosterheld and Mc

Naughton 1988; Cesaroli et al. 2004), growth following defoliation in this experiment produced leaves of higher SLA, though differences with the control plants were generally not significant. The higher SLA of new foliage following defoliation may be a response to maximise the leaf area available for light capture for every unit of biomass invested (James and Bell 2000). While higher levels of incident light are normally associated with smaller SLA in lower crown zones (Pinkard and Beadle 1998a), defoliation in this experiment had no significant effect on SLA of leaves in lower crown zones.

This study is rejected the hypothesis that defoliation increased foliar nitrogen content. Indeed, there were no significant changes in foliar nitrogen following defoliation. Also, there was also no evidence that defoliation changed the relationship between A_{\max} and foliar nitrogen content, as has been found in some studies (Lovett and Tobiessen 1993; Ovaska et al. 1993b; Reich et al. 1993; Volin et al. 2002). A few studies have found a correlation between up-regulation of photosynthesis and increased foliar nitrogen content as a result of change in the allocation of nutrient (Hoogesteger and Karlsson 1992; Morrison and Reekie 1995; Lavigne et al. 2001). Defoliation had no significant effect on foliar carbohydrate concentrations, and there was no clear pattern of carbon storage.

Numerous studies have attributed increased photosynthetic rates following defoliation to increased stomatal conductance, g_s (Reich et al. 1993; Morrison and Reekie 1995), possibly resulting from improved plant water status (Trumble et al. 1993; Pataki et al. 1998). In this study, maximum g_s was higher in defoliated than control trees irrespective of the crown zone; compensatory response in water use of defoliated trees was reported at the experimental site (see Chapter 6). A possible mechanism for increases in g_s following defoliation may be increased soil-to-leaf hydraulic conductance (K_p). It has been reported previously that defoliation may result in increased K_p (Salleo et al. 2003). Numerous studies have also demonstrated that g_s varies linearly with K_p , indicating that changes in plant water status may affect plant productivity (Meinzer and Grantz 1990; Hubbard et al. 2001; Brodribb et al. 2002). Up-regulation of photosynthesis of defoliated trees in the current experiment may also be associated with the increased K_p .

In conclusion, the largest and most enduring increases in photosynthesis following partial defoliation in 4-year-old field-grown *E. globulus* were observed in the lower outer crown foliage, although up-regulation also occurred for a shorter period in the newly-formed foliage of the upper crown. Despite the positive relationship between foliar nitrogen content and A_{\max} , foliar nitrogen content was not responsible for the photosynthetic enhancement. Stomatal conductance possibly combined with increased light absorption appeared to be the major traits driving the photosynthetic up-regulation in defoliated trees. The results also suggest the idea of improvement in tree water status.

Chapter 6. Responses of transpiration and canopy conductance.

6.1 INTRODUCTION

Removal of significant amounts of leaf material by herbivory or via artificial defoliation may alter the water balance within trees and forests significantly by reducing transpiration losses (Buckhouse and Colthorp 1976; McNaughton 1979; 1983; Cunningham et al. 2009). Water transport and photosynthesis in trees is regulated by the hydraulic conductance of the pathway from the soil-to-leaf (Tyree 2003) and by leaf area (Mencuccini 2003). Thus defoliation and the associated changes in leaf area could significantly affect whole-plant hydraulic conductance. In a study of defoliation by gypsy moth on the water balance of deciduous forest trees, Stephens et al. (1972) showed that leaf water potentials were higher in defoliated trees than those that were not defoliated. Similarly, Reich et al. (1993) observed that defoliated red pine (*Pinus resinosa* Ait) trees had higher transpiration rates than nearby non-defoliated trees. It has also been observed that partial defoliation may result in increased soil-to-leaf hydraulic conductance (K_p) (Salleo et al. 2003).

Plant hydraulic conductance is important in the regulation of photosynthesis and stomatal conductance within canopies (Teskey et al. 1983; Meinzer and Grantz 1990; Pataki et al. 1998; Hubbard et al. 1999) and several studies have shown that stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance, indicating that changes in plant hydraulic properties may affect plant productivity (Hubbard et al. 2001; Brodribb et al. 2002). Little is known about the effect of defoliation on water use by large trees; and to my knowledge no studies have examined this in eucalypts. In the current study, I investigated diurnal patterns of transpiration and canopy conductance in four-year-old defoliated and non-defoliated *E. globulus* trees growing under similar atmospheric conditions in an irrigated plantation.

I tested the hypothesis that water relations of *E. globulus* would improve following a partial defoliation and addressed the following questions:

1. does defoliation result in improved water status as measured by pre-dawn and midday leaf water potential?
2. does defoliation affect rates of transpiration and canopy conductance?
3. does partial defoliation result in an increase in soil-to-leaf hydraulic conductance?

6.2 MATERIALS AND METHODS

Site and biophysical parameters are described in the Chapter 2. Defoliation treatment is described in Chapters 4 and 5.

6.2.1. *Tree water use*

Sap flow was measured at 1.3 m height on each of the six trees using SF100 sap-flow probes and loggers (Greenspan, Warwick, Qld., Australia). Probesets were inserted radially into the trunk on the northern and eastern aspects, and thermistors were stratified with depth. Thus, heat-pulse velocity was measured at four depths across the sapwood. Heat-pulse velocity was estimated at 15-min intervals for the duration of the experiment. Heat-pulse velocity was converted to sap velocity (J_s) following Edwards and Warwick (1984) and scaled to tree water-use using the weighted averages technique (Hatton et al. 1990). The solutions of Swanson and Whitfield (1981) were applied to correct for the effects of wounding using a wound width of 3.1 mm (O'Grady et al. 2008). Bark depth, sapwood depth and heartwood depth were determined from core samples taken from each tree. There was no heartwood present in these trees and this was confirmed using dimethyl orange dye. Thus it was assumed that all sapwood was conducting. Leaf area of each tree was estimated using allometric

equations developed for trees at this site (O'Grady et al., 2006). Relationships between sapwood area or leaf area and time were determined by linear regression for control and defoliated trees and growth coefficients were used to calculate tree water use (Table 1). Water use was expressed on a per tree basis as either Q (sapwood area) or E (leaf area).

6.2.2. Canopy conductance

Canopy conductance (G_C) was calculated from transpiration per unit leaf area (E) following Ewers et al. (2005) where:

$$G_C = \frac{K_G(T_A)E}{D} \quad (\text{Eq. 6.1})$$

where $K_G(T_A)$ is the conductance coefficient as a function of temperature (115 ± 0.4236 (T_A ; °C); $\text{kPa m}^3 \text{ kg}^{-1}$) which accounts for the temperature effects on the psychrometric constant, latent heat of vaporisation, specific heat of air at constant pressure and the density of air (Phillips and Oren 1998). T_A is air temperature, E is transpiration rate per unit leaf area and D is vapour pressure deficit. Canopy conductance was converted from m s^{-1} to $\text{mmol m}^{-2} \text{ s}^{-1}$ using equations in Pearcy et al. (1989). Estimates of canopy conductance calculated in this manner assume that boundary layer conductance is high so that D is close to leaf-to-air vapour pressure deficit. It also assumes no vertical gradients in D through the canopy and negligible water storage above the sap flux measurement point. Eucalypts in general are strongly coupled to the atmosphere (Morris et al. 1998; Mielke et al. 1999; Hutley et al. 2000; Whitehead and Beadle 2004), and these assumptions hold for the trees at this site (O'Grady et al. 2008). Furthermore, to keep the measurement errors in G_C below 10%, G_C was calculated only when $D \geq 0.6 \text{ kPa}$ (Ewers and Oren 2000). Estimates of E and G_C were made between 29/08/06 and 01/04/07.

6.2.3. Leaf water potential

Pre-dawn and midday leaf water potentials (Ψ_l) were measured using a Scholander-type pressure chamber on four occasions after the application of the defoliation treatment: 06/10/06, 14/12/06, 22/01/07, and 26/03/07. Pre-dawn leaf water potential (Ψ_{pd}) was measured between 0330 and 0530 h AEST and midday leaf water potential (Ψ_{md}) was measured between 1130 and 1330 h AEST. For each measurement, four leaves newly-produced were excised from the upper crown of each tree on the northern aspect. Collected leaves were immediately placed into plastic bags within an insulated ice-cooled container to prevent transpiration prior to measurement, which was usually completed within 15–20 min of collection.

6.2.4. Soil-to-leaf hydraulic conductance

Soil-to-leaf hydraulic conductance (K_p) was calculated from the relationship between transpiration and water potential following Loustau and Granier (1993):

$$K_p = \frac{E_{md}}{(\Psi_{pd} - \Psi_{md})} \quad (\text{Eq. 6.2})$$

Where E_{md} is midday transpiration per unit leaf area and Ψ_{pd} and Ψ_{md} are pre-dawn and midday leaf water potential, respectively.

6.2.5. Model development

The average daily canopy conductance (G_C) was plotted against the average daylight air temperature (T), vapour pressure deficit (D) and daily total solar radiation (R) for each treatment. With the aim of explaining canopy conductance and plant transpiration responses throughout the year, mathematical functions were fitted to the data, to find consistent relations between G_C and each variable, using the maximum boundary-line analysis (Jones, 1994; Webb, 1972). The boundary line analysis was used to represent

the upper limit of the scattering in the diagrams and to indicate the hypothetical response of each independent variable when the other independent ones are not limiting (Jones, 1994). The boundary lines were fitted by eye determining the maximum G_C values in the dispersion plots with T , D and R . The fitting of the boundary line required the subdivision of x -axes into artificial groups. Outliers were removed if they were considered to not fit the observed trend using the Dixon's test at a 95% confidence level. The best fit for air temperature (T) boundary line was a second degree polynomial equation (Figure 6.1a). An exponential decay function was fitted to a plot of G_C and D (Figure 6.1b). The best fit of G_C in relation to solar radiation (R) was a non-rectangular hyperbola presented in figure 6.1c.

From this data, a phenomenological model of the stomatal conductance was developed (White et al., 1999):

$$G_C = G_{C \max} f(T)f(D)f(R) \quad (\text{Eq. 6.3})$$

When predicted G_C was fitted to observed values for the undefoliated trees by linear regression, the model explained 91% of variation in G_C of *E. globulus* (Figure 6.2).

6.2.6. Statistical analysis

Repeated-measures ANOVA were used to test for differences in leaf water potential, daily maximum transpiration rates (E_{\max}) and daily maximum conductance ($G_{C\max}$), and soil-to-leaf hydraulic conductance (K_P) between undefoliated and defoliated treatments, assuming a significance level of 0.05, using Genstat software (GENSTAT Committee, 1989). A group regression procedure (McPherson, 1990) was used to determine whether the effect of defoliation treatment significant affected the slope and/or intercept of the relationship between Ψ_{md} and E_{\max} , and G_C and the atmospheric variables using Genstat10 software. This procedure tests the hypotheses that (1) the regression lines have common slope allowing for the possibility that they have different intercepts, and (2) that the same line applies to all defoliation treatments.

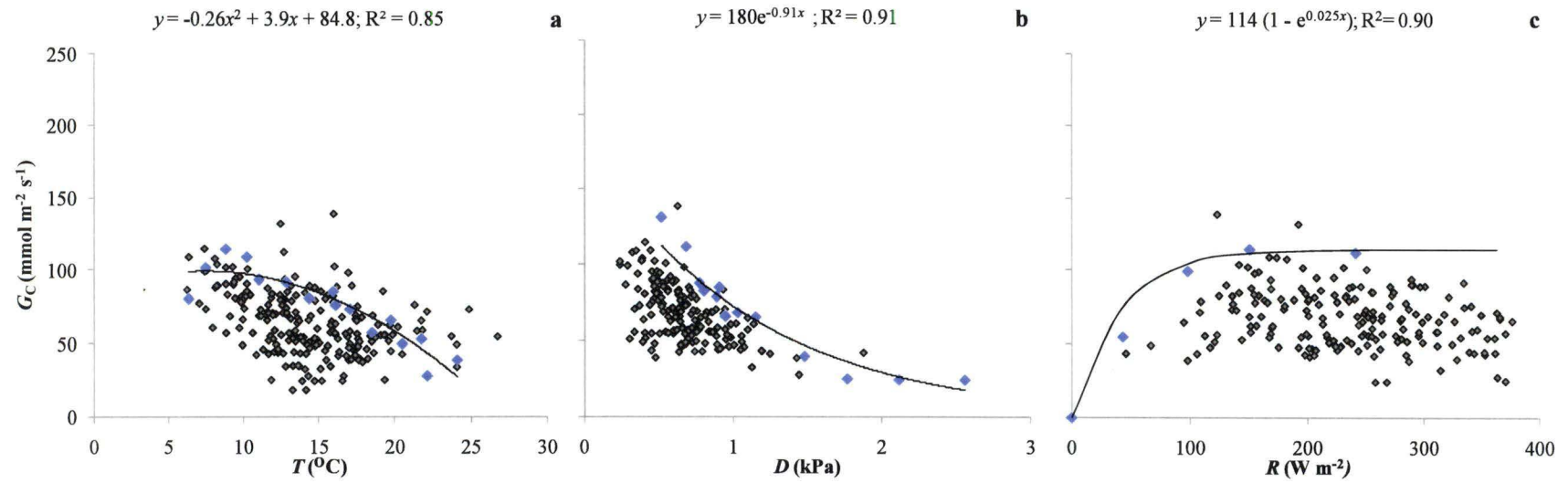


Figure 6.1. Scatter diagrams of the measurements of canopy conductance (G_C) in the undefoliated treatment plotted against (a) air temperature (T), (b) air vapour pressure deficit (D) and (c) solar radiation (R), with the hypothetical boundary line fitted for each environmental variable.

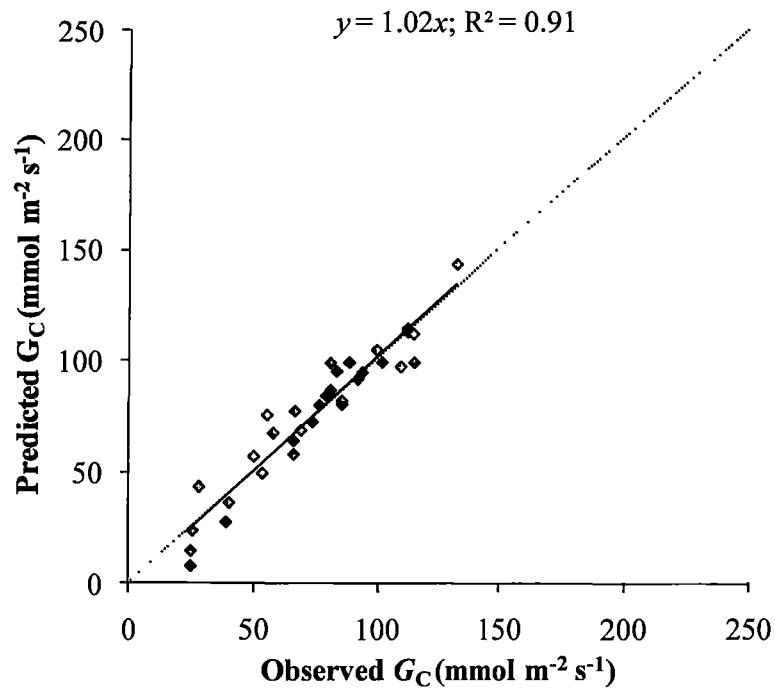


Figure 6.2. Predicted versus observed canopy conductance (G_C) of *E. globulus* in the undefoliated treatment. The line is 1:1.

6.3. RESULTS

6.3.1. Climate and soil matric potential

Total rainfall during the study period was 245 mm (Figure 6.3a), 20% less than the average for the same period between 1995 and 2005 (Australian Bureau of Meteorology, www.bom.gov.au). The wettest month was Jan 07 with 84 mm; the driest months were Nov 06 and Mar 07 with only 15 and 16 mm of rainfall respectively. Sep, Oct, Dec 06 and Feb 07 averaged 32 mm rainfall month⁻¹. Over the 215 days of the study, the trees received approximately 545 mm of water via irrigation; pan evaporation for this period was 1090 mm (Australian Bureau of Meteorology, www.bom.gov.au).

Monthly maximum temperature (T_{\max}) and maximum vapour pressure deficit (D) are illustrated in Figures 6.3b and 6.3c, respectively. Maximum T was recorded in Feb and Mar 07 (summer) with an average around 25°C. The coldest months were Sep and Nov 06 (spring) with low minimum and maximum temperatures. Maximum D was highest in Oct 06 and Feb 07 (around 2.4 kPa), intermediate in Dec 06, Jan and Mar 07 (around 1.6 kPa) and lowest in Sep and Nov 06 (around 0.9 kPa).

Soil matric potential remained high throughout the study period. At the beginning of the study, matric potential of shallow soils (0–100 mm) was approximately -0.03 MPa and increased with depth to approximately -0.005 MPa at about 2 m. Irrigation commenced in spring (Oct 06) and matric potential of shallow soils increased to -0.01 MPa (Figure 6.4). By mid-summer (Jan 07), soil water potential of shallow soils (0–100 mm) was < -0.2 MPa and by autumn (Mar 07) was -0.34 MPa (Figure 6.4). In the deepest soil horizons (>100 mm), soil water availability was relatively uniform throughout the soil profile and across measurement dates (Figure 6.4).

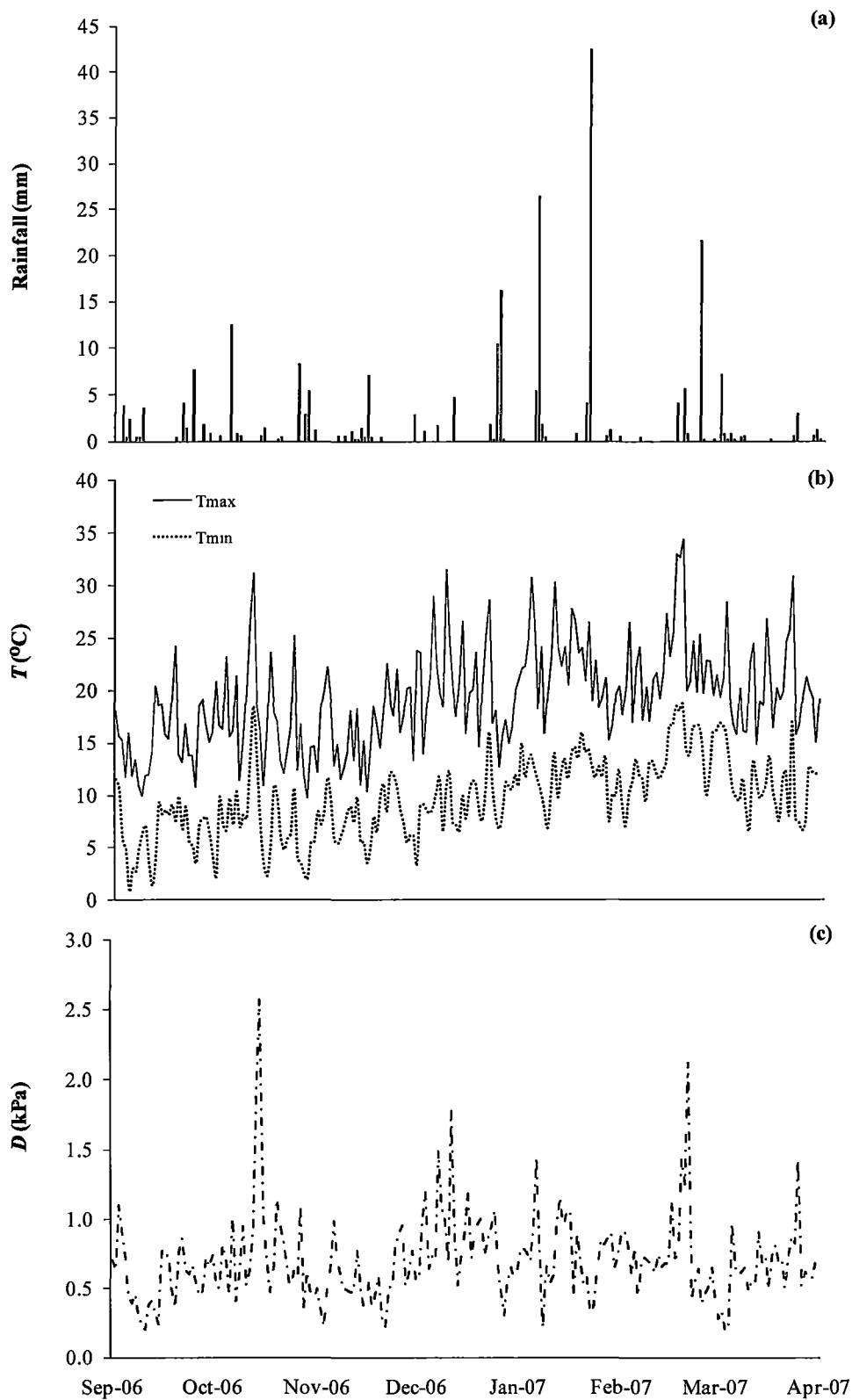


Figure 6.3. Daily variation in (a) rainfall, (b) air temperature (T) and (c) daily vapour pressure deficit (D) in *E. globulus* plantation in Southern Tasmania over the growing season of 2006/2007.

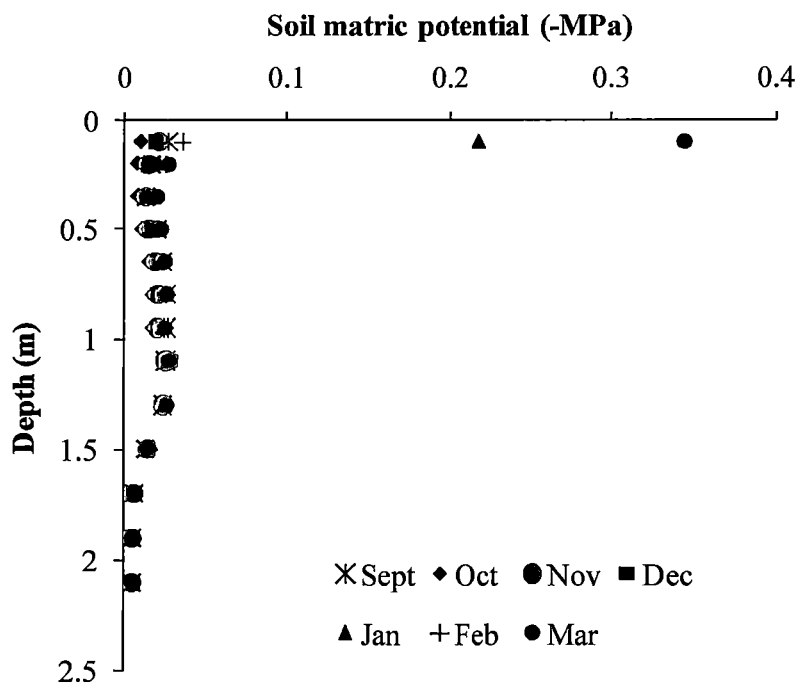


Figure 6.4. Typical profiles of soil matric potential in the experimental plot. Measurements were made on seven occasions. Irrigation commenced in early October 2006.

6.3.2. Leaf water potential

Pre-dawn leaf water potential (Ψ_{pd}) varied significantly by day, but not by defoliation treatment or the day-by-treatment interaction (Table 6.1). Minimum Ψ_{pd} was -0.47 ± 0.18 MPa in early summer (Dec 06). There was no significant differences in Ψ_{pd} between defoliated and control trees ($P > 0.1$; Figure 6.5). Midday leaf water potential of the control trees was lowest on 06/10/06 (-1.80 ± 0.67 MPa) and highest in mid-summer (Jan 07) (-1.23 ± 0.38 MPa) ($P < 0.001$). There were no significant differences in Ψ_{md} between defoliated and control trees in early summer and mid-summer (Figure 6.5), however Ψ_{md} was significantly higher in defoliated than control trees in spring (Oct 06) and at the end of summer (Mar 07) ($P < 0.1$; Figure 6.5).

Table 6.1. Summary of repeated-measured ANOVA on day basis showing degrees of freedom (*Df*), *F* and probability (*P*) and standard error (*SED*) for each analysis.

Parameters	Factors	<i>Df</i>	<i>F</i>	<i>P</i>	<i>SED</i>
Ψ_{pd} (MPa)	Day	3	15.14	0.001	0.19
	Treatment	1	1.08	0.309	0.18
	Day×treatment	3	2.73	0.104	0.29
Ψ_{md} (MPa)	Day	3	46.26	0.001	0.54
	Treatment	1	8.55	0.008	0.45
	Day×treatment	3	7.36	0.001	0.80
Q (mmol m ⁻² day ⁻¹)	Day	215	9.01	0.008	2.07
	Treatment	1	0.52	0.510	3.65
	Day×treatment	215	1.02	0.402	4.68
E_{max} (mmol m ⁻² s ⁻¹)	Day	215	7.19	0.029	0.007
	Treatment	1	7.15	0.055	0.012
	Day×treatment	215	1.38	0.309	0.016
G_{Cmax} (mmol m ⁻² s ⁻¹)	Day	215	6.44	0.064	9.29
	Treatment	1	22.35	0.001	16.74
	Day×treatment	215	2.66	0.117	21.26
K_p (mmol m ⁻² s ⁻¹ MPa ⁻¹)	Day	3	18.33	0.001	0.004
	Treatment	1	9.44	0.037	0.007
	Day×treatment	3	2.08	0.174	0.009

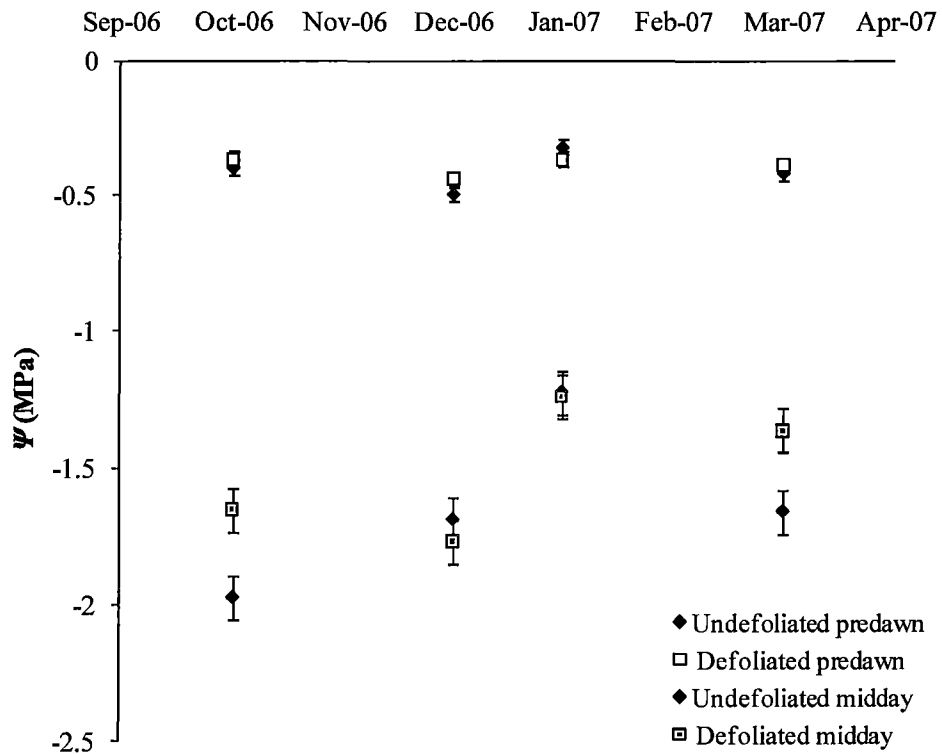


Figure 6.5. Mean predawn and midday leaf water potential (Ψ) of undefoliated and defoliated *E. globulus*. Data are presented for four measurement days: Oct 06, Dec 06, Jan 07, and Mar 07. Error bars show standard error ($\alpha=0.05$).

6.3.3. Transpiration and canopy conductance

In contrast to expectations and despite defoliation, differences in daily transpiration per unit sapwood area (Q) between undefoliated and defoliated treatments were not significant ($P>0.1$; Table 6.1). However there were marginal differences between both treatments in maximum transpiration rates (E_{\max}) and canopy conductance ($G_{C\max}$) ($P<0.1$; Table 6.1), although E_{\max} and $G_{C\max}$ varied considerably from day-to-day. Trees in both treatments showed a positive strong correlation between midday leaf water potential and maximum transpiration rates (Figure 6.6). However, the defoliation treatment significantly affected the intercept of the relationship ($P<0.1$), but not the slope ($P>0.1$).

Diurnal patterns of E and G_C for two contrasting days in terms of D are illustrated in Figure 6.7. At the end of winter (Sep 06), D did not exceed 1.0 kPa (Figure 6.7e), whereas it reached 2.35 kPa at 1600 AEST 52 days later (spring; Oct 06) (Figure 6.7f). Changes in the diurnal patterns in response to defoliation were visible between these two days. At the end of winter, shortly after the defoliation treatment, there were no significant differences in E and G_C between undefoliated and defoliated treatments ($P>0.1$; Figure 6.7a and 6.7c). Both E_{\max} and $G_{C\max}$ were recorded simultaneously at 1230 and 1300 AEST for the control trees and defoliated trees, respectively. In spring, defoliated trees exhibited significantly higher E and G_C between 0900 and 1630 AEST and 1100 and 1600 AEST, respectively, than the undefoliated trees ($P<0.05$; Figure 6.7b and 6.7d). In undefoliated trees, the maximum value of G_C was reached at D of 1kPa (Figure 6.7f), and then declined throughout the day (Figure 6.7d); in defoliated trees, G_C reached its maximum at D of 2.35 kPa (Figure 6.7f) before declining.

6.3.4. Soil-to leaf hydraulic conductance

There were significant differences in hydraulic conductance (K_P) between treatments ($P<0.1$; Table 6.1). Overall, K_P was lower for undefoliated than defoliated trees (Figure 6.8). There was no significant difference in K_P between treatments in spring

(Oct 06), but on the three following measurement days K_p of defoliated trees was significantly higher than K_p of the undefoliated trees ($P < 0.05$; Figure 6.8).

6.3.5. Canopy conductance and atmospheric variables relationships

For defoliated treatment, $f(T)$, $f(D)$ and $f(R)$ were also fitted to the upper boundary of normalised plots of G_C against T , D and R respectively (Figure 6.9). When the upper canopy of *E. globulus* trees was defoliated, the optimum values of G_{Cmax} were increased in the sub-models compared to the control (Figure 6.9). An optimum air temperature of 10.9°C leading to the maximum G_C of defoliated trees under the experimental conditions is derived from the fitted equation (Figure 6.9a). However, the defoliation treatment did not change the intercept or the slope of the relationship established by the phenomenological model compared to the control treatment ($y = 0.99x$; $R^2 = 0.95$; $P < 0.05$; Figure 6.10).

6.4. DISCUSSION

Throughout the course of this study, pre-dawn and midday leaf water potentials were well above those associated with turgor loss (-1.8 MPa) in *E. globulus* (White et al., 1996). Although pre-dawn leaf water potential of the residual leaf area remained similar to control levels, higher midday leaf water potential in defoliated *E. globulus* trees was associated with increased maximum transpiration per unit leaf area (Figure 6.6). These results are consistent with previous studies where an increase in leaf water potentials in response to defoliation has been observed (Osman and Sharrow, 1993; Stephens et al., 1972; Vanderklein and Reich, 2000; Welker and Menke, 1990). The lack of a significant increase in pre-dawn leaf water potential following defoliation may be related to the frequency that water was added through irrigation to the experimental plot.

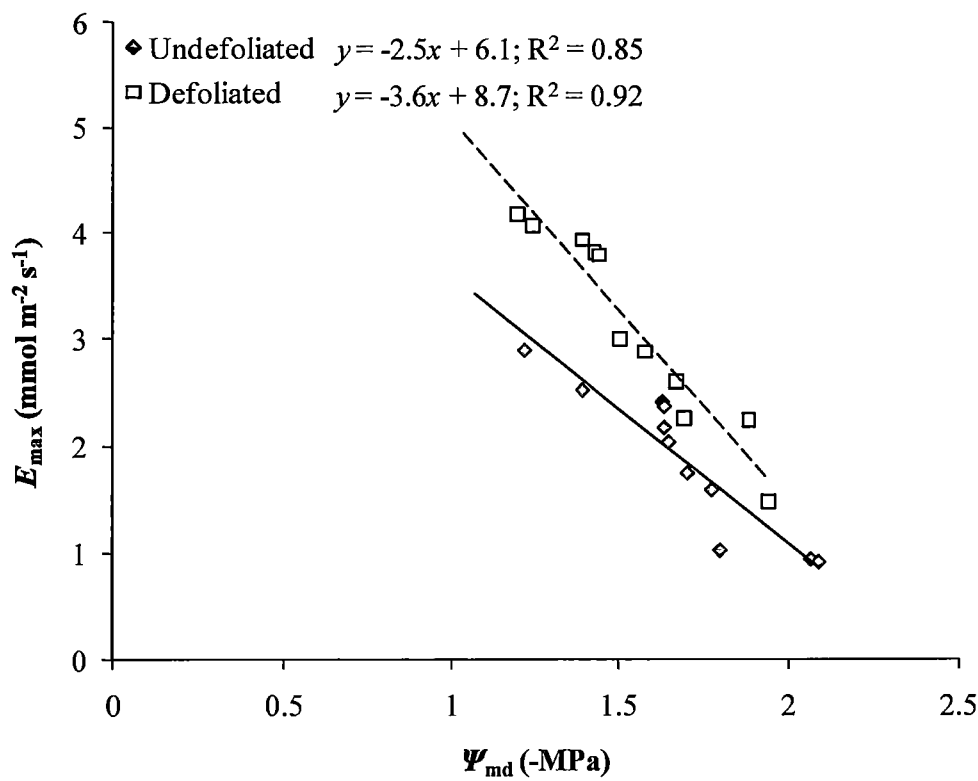


Figure 6.6. The relationship between maximum rates of transpiration (E_{\max}) with midday leaf water potential (Ψ_{md}) for undefoliated and defoliated *E. globulus* trees growing under similar atmospheric conditions.

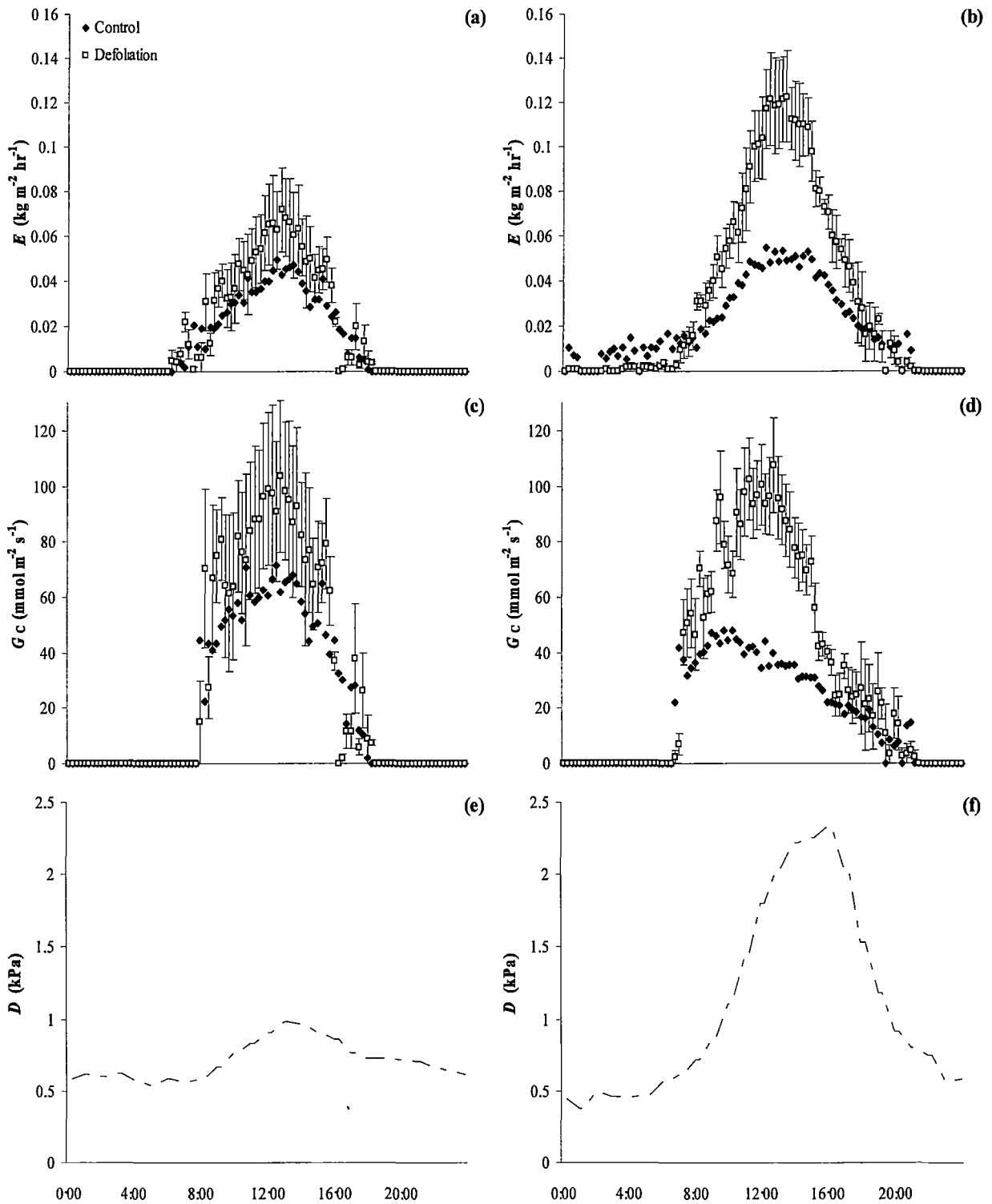


Figure 6.7. Diurnal courses of transpiration (E , a and b), canopy conductance (G_c , c and d) in control and defoliated *E. globulus* trees on two contrasting days: 02/09/2006 (a,c,e), and 24/10/2006 (b,d,f) with respect to vapour pressure deficit (D , e and f). Data represent the mean and standard error of three trees ($\alpha=0.05$). For clarity, error bars are presented only for control trees.

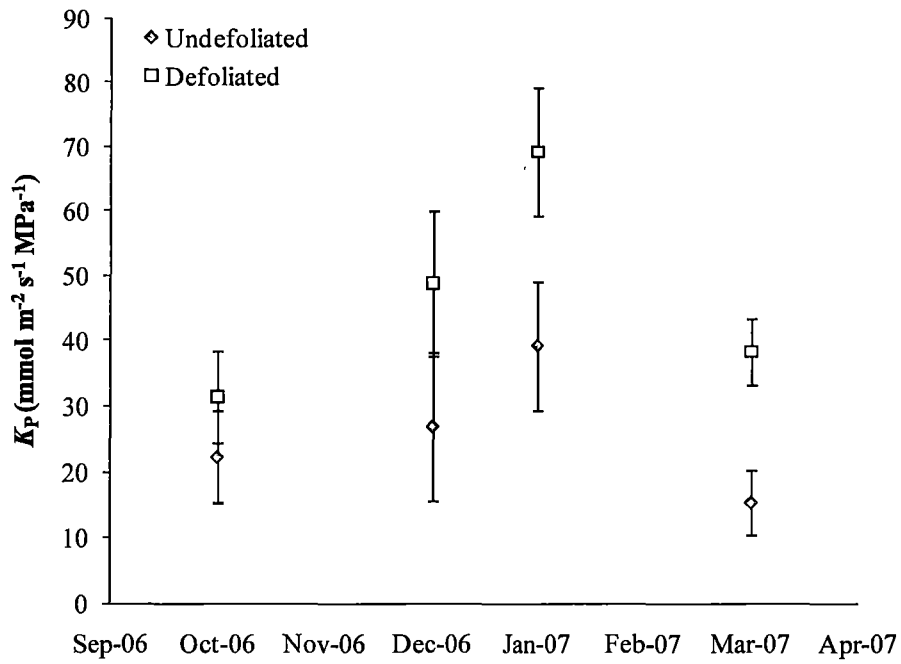


Figure 6.8. Mean soil-to-leaf hydraulic conductance (K_p) of control and defoliated *E. globulus* trees in southern Tasmania. Data are presented for four occasions. Error bars show standard error ($\alpha=0.05$).

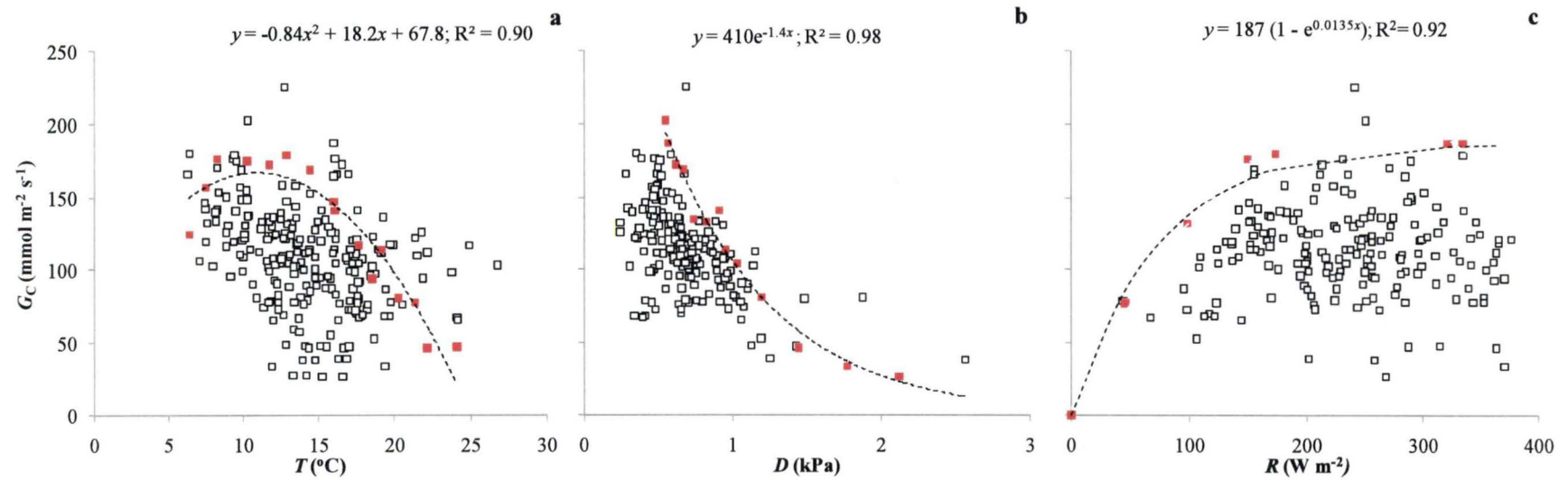


Figure 6.9. Scatter diagrams of the measurements of canopy conductance (G_C) in the control and defoliated treatments plotted against (a) air temperature (T), (b) air vapour pressure deficit (D) and (c) solar radiation (R), with the hypothetical boundary line fitted for each environmental variable.

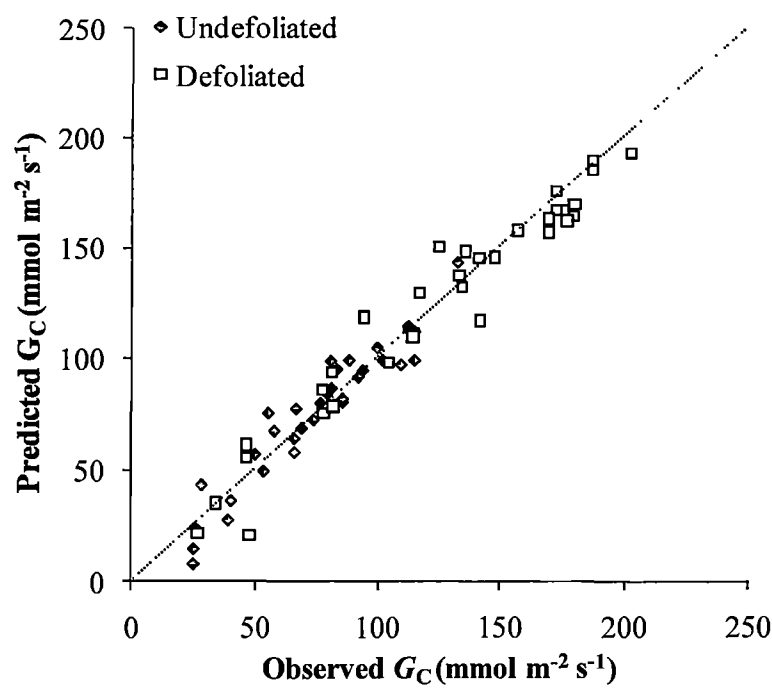


Figure 6.10. Predicted versus observed canopy conductance (G_C) of *E. globulus* in the control and defoliated treatments. The line is 1:1.

Tree leaf area is an important determinant of whole-plant transpiration (Roberts, 2000). Following the removal of foliage from the upper canopy, defoliated trees had higher transpiration rates and canopy conductance than control non-defoliated trees, providing strong support for hydraulically mediated control of gas exchange (Meinzer and Grantz, 1990; Oren et al., 1999; Pataki et al., 1998). Meinzer and Grantz (1990) increased K_P in sugar cane experimentally by partial defoliation. In that study, removal of 40% of plant leaf area resulted in increased leaf stomatal conductance, such that E on a whole-plant basis, and leaf water potential of the residual leaf area remained similar to pre-defoliation levels. Similarly, in a companion study measuring the defoliation effect on leaf-level CO_2 assimilation on the same site and with the same trees, increased photosynthetic rate was correlated with increased stomatal conductance (g_s) throughout the tree crown (see Chapter 5). Pataki et al. (1998) observed increased canopy conductance in defoliated *Pinus taeda* L. trees and also attributed this to increased soil-to-leaf hydraulic conductance. In the current experiment, increased E and G_C could be attributed, at least in part, to increase in K_P , as K_P was higher in the defoliated than control trees throughout the experiment.

Supply of water from the soil-to-leaf places a major constraint on leaf gas-exchange. Strong correlations between the soil-to-leaf hydraulic conductance and g_s or E have been reported in a number of studies (Brodribb and Holbrook, 2004; Lo Gullo et al., 2005; Meinzer and Grantz, 1990; Reich and Hinckley, 1989; Sperry, 2000; Teskey et al., 1983). It is generally accepted that increases and decreases in K_P result in similar directional changes in stomatal conductance (Brodribb and Holbrook, 2004; Teskey et al., 1983). Thus, higher soil-to-leaf hydraulic conductance and the resultant increases in stomatal conductance observed in the companion study (see Chapter 5) provide a mechanistic insight into the commonly observed up-regulation of photosynthesis (Pinkard et al., 1998; Reich et al., 1993; Turnbull et al., 2007) in response to defoliation, as increased K_P alleviates leaf water stress, permitting increased conductance to both water vapour and CO_2 .

Although no significant differences in whole tree water use between the two treatments were observed in this study, defoliated trees experienced a short term decrease in water flux (Q) shortly after the application of the defoliation treatment. However, as the

experiment progressed and leaf area recovered (Table 6.1), rates of water use in the defoliated trees were similar to that observed in the control trees. This is a significant result, as it is often assumed that defoliation reduces transpiration (Cunningham et al., 2009), potentially having a significant impact on forest water balance. In Cunningham et al. 2009, the reduction in transpiration rates was in response to alleviation of natural defoliation via application of insecticide and not imposed by a defoliation event. Also, leaf damage was attributable to an outbreak of scale insects, which are recognised to inhibit phloem transport or the opening of stomatal aperture (Peterson and Higley, 1993). On the contrary, the present study presented the recovery response to a single imposed defoliation event, and the expected reductions in water use might have been offset by the recovery in leaf area and/or increases in soil-to-leaf conductance and associated increases in gas exchange, such that total volumes of water transpired remained similar in the two treatments.

At the stand scale, regulation of water use may be modified considerably by the structure of the vegetation, which influences coupling with the microclimate or soil moisture, and may result in maintenance of forest transpiration within tightly controlled limits (Phillips and Oren, 2001; Roberts, 1983; Roberts, 2000). For example, Honeysett *et al.* (1992) found similar transpiration rates in two species of eucalypts (*Eucalyptus nitens* and *E. delegatensis*) under conditions of similar water availability and atmospheric conditions even though the leaf area index of the *E. nitens* stand was twice that of the *E. delegatensis* stand. In the current study, decoupling between leaf area and water flux might explain the similarity in Q between the two treatments. White *et al.* (2000) reported that perfect coupling was not valid when the leaf area index is high, as it was in this study, in an *E. globulus* plantation. Despite the considerable progress made during the last three decades in the knowledge of plant transpiration rate, understanding the underlying extrinsic and intrinsic regulatory mechanisms of forest water use are still incomplete.

At the canopy level, this model strongly resembles similar phenomenological models developed for *E. nitens* and *E. globulus* at the leaf level (White et al., 1999). In the current study, the variation in observed G_C explained by the model was unaffected by the defoliation treatment. Increased stomatal regulation of water use in response to

increased atmospheric D plays an important role in conserving water and maintaining water status within limits that avoid catastrophic loss of xylem function. In this study, on a day of high atmospheric demand, G_C peaked mid-morning. Typically, G_C in these trees peaked at D at 1 kPa, and declined steadily throughout the day. In contrast, E closely followed diurnal patterns in D in both control and defoliated treatments, although the decline in E occurred later in the day in defoliated trees than in the control trees. Transpiration of defoliated trees was not limited by stomatal closure but depended on D . This result suggests that defoliated trees were less sensitive to high atmospheric D presumably because more water was available per unit leaf area, resulting from higher soil to leaf conductance.

Understanding of the regulation of plant water use is an important consideration for managing plantations and their associated impacts on water balance. Defoliating agents such insects can significantly affect plantation productivity but their effect on plantation water use remains poorly understood. This study has shown that experimental manipulation of plant canopies can be a useful tool for elucidating the controls on plant water-relations and for predicting the responses of plantations to defoliation. Furthermore there remains considerable concern about the environmental effects of plantation forestry using fast growing tree species on water catchments. Our study has shown that although leaf area declined significantly in response to defoliation, defoliation resulted in higher soil-to-leaf conductance leading to increased G_C and E in defoliated trees compared to control trees. These results contradict prevailing paradigms that defoliation could significantly reduce plantation water use (e.g. Cunningham et al., 2009) but do provide strong support for strong hydraulic regulation of gas exchange in plant canopies.

Chapter 7. Interactive effects of water supply and defoliation.

7.1. INTRODUCTION

Plants have evolved sophisticated strategies to defend against abiotic and biotic stressors. Stressors are external factors that exert a detrimental influence on plant performance. Stress is a deviation in plant performance, compared to that of controls, in response to a specific change in growing conditions. The ability of plants to adapt to stress relies on resistance mechanisms that allow their tissues to avoid, tolerate, or even acclimate to the consequences of stressors (Karban and Baldwin 1997). Resistance mechanisms are driven by either structural or physiological adjustments, or by a combination of both. Individually, stressors such as water deficit, salinity, heat or pests have been the subject of intense research (Karban and Baldwin 1997; Munns 2002). It has been shown that generally, plants respond to abiotic or biotic stressors by alteration of carbon and nutrient metabolism, which in turn influences plant carbohydrate production and secondary chemistry (Bryant et al. 1983).

In natural environments, trees are routinely subjected to a combination of different biotic and/or abiotic stressors. Among them, insect defoliation and low water availability are common causes of tree dieback (Hogg et al. 2002; Bréda and Badeau 2008) and loss of productive potential in eucalypt plantations in Australia (Nahrung 2003; Mummery and Battaglia 2004). In the literature, considerable evidence points out that drought stress promotes outbreaks of insect defoliators (Landsberg, 1985; Schowalter et al., 1999; Rouault et al., 2006; Netherer and Schopf, 2010). The evidence associating insects and drought is incidental, consisting of observations that outbreaks of insects are typically preceded by drought episodes (Wargo 1996). Drought may enhance the attractiveness or acceptability of plants to insects, make plant tissues more suitable for insect growth, survival, and reproduction, or enhance the ability of insects

to detoxify plant defensive chemicals and thus lead to outbreaks (Mattson and Haack, 1987).

The “plant stress” hypothesis proposes that plants under abiotic stress become more suitable as food for herbivorous insects, and thus decrease individual plant resistance to insect herbivory (White 1974; 1984; Mattson and Haack 1987). Indeed, insect herbivores frequently reach outbreak densities on plants associated with environmental stress (e.g. Louda and Collinge 1992; Kirschbaum et al. 2007; Gorsel van et al. 2008). It is widely predicted that changing climates will affect the amount and distribution of rainfall (Allen and Ingram 2002), which may also affect pest populations and outbreaks (Ayres and Lombardero 2000; Netherer and Schopf 2009). Across much of the main plantation growing regions of southern Australia annual rainfall has been declining since the 1970’s (www.bom.gov.au), potentially resulting in an increased frequency of low water availability for the trees and an associated loss of production. The response to co-occurrence of multiple stressors is not usually predictable from single-factor analyses. This makes the study of interactions both appropriate and complex., as a combination of stressors can result in intensification, overlapping or reversal of the stress effects (Osmond et al. 1987). Resolving these potential outcomes is essential for predicting the impact of stressors on forest production.

Dendrochronological analyses in North American (Hogg et al. 2002) and European forests (Bréda and Badeau 2008) have been used to provide evidence of multiple stressors affecting forest growth. Parallel studies with *Quercus robur* and *Q. petraea* have used ecophysiological traits to investigate the effects of multiple stressors on morphological, anatomical and physiological responses (Gieger and Thomas 2002; Gieger and Thomas 2005). Gieger and Thomas (2005) found that stomatal conductance of *Q. robur* was primarily regulated by the tree’s capability of transporting water to the leaves, i.e. increased hydraulic conductance, when defoliation and drought were applied within the same growing season. In contrast, stomatal conductance of *Q. petraea* was mostly determined by the environmental variable (atmospheric vapour pressure deficit). Stomatal conductance is a key variable in the regulation of the plant’s water use, and it responds in opposite ways upon the influence of drought or defoliation. Stomatal conductance is responsive to two distinctive environments: the aerial environment of the

leaf, which is defined by irradiance, temperature, humidity, CO₂ concentration and boundary-layer condition; and the environment that mediates water supply and induces chemical (via abscisic acid) and/or physical signals (via hydraulic conductivity; e.g. Tyree 2003) of water stress (e.g. Mencuccini 2003). Stomatal conductance also responds to leaf water potential, which is influenced by the aerial environment via transpiration (e.g. Jones 1998). It is generally acknowledged that stomatal conductance is affected by the atmospheric water vapour pressure deficit, with stomatal conductance being related to vapour pressure deficit by a logarithmic hyperbolic decay (see Chapter 6; Oren et al. 1999).

Growth models draw upon knowledge gained from physiological research conducted on many aspects of plant growth and prove to be a useful way of exploring uncertainty through testing different hypotheses of system interactions over a long term period (Pinkard et al. 2009a; 2009b). In recent study, models consistently predicted that under various climate change scenarios, disturbance from defoliating insects and foliar pathogens would be detrimental to eucalypt forest primary net productivity (Pinkard et al. 2009b). With increasing use of growth models in operational management forestry, a proper model validation is a primary concern to achieve simulation process and sustainable management decisions. Process-based models of plant growth provide a useful framework for determining how leaf removal by defoliating agents influences processes regulating growth. The predictions by CABALA (from CARbon BALAnce) has been validated for examining the effects of *Mycosphaerella* leaf diseases (MLD) on rotation-length *E. globulus* plantation productivity under current and future climates, for the range of MLD levels applied (Pinkard et al. 2009a).

To date, studies of physiological responses of *E. globulus* have focused on a single stressor (Pereira et al. 1987; White et al. 1996; Pinkard et al. 2007; Turnbull et al. 2007; O’Grady et al. 2008). Currently there is no information about physiological responses of eucalypt species to multiple stressors in a field environment. In this study, I investigated the interacting effects of low water supply and artificial defoliation on the gas exchange, water relations and growth of *E. globulus* saplings. I asked the following questions: Does defoliation improve the water status of plants grown under low water availability? If so, does this response provide a mechanistic underpinning of the often observed up-

regulation of stomatal conductance and photosynthesis and what are the implications for forest plantations? The young saplings were grown in rain-fed, representing low water availability and irrigated, representing high water availability, conditions. The working hypotheses were:

1. defoliated *E. globulus* were able to compensate for the loss of foliage, i.e. increased photosynthetic rates and stomatal conductance following defoliation, and this irrespectively of the watering treatment;
2. the physiological response was regulated by the tree's capability of transporting water to the leaves;
3. defoliation offsets the negative effects imposed by the rain-fed treatment on growth.

The potential use of CABALA to predict the impacts of defoliation was tested on *E. globulus* diameter growth (d , mm), entire stem volume (V , m³ ha⁻¹) and cumulative daily water use (Q , mmday⁻¹) under the different watering conditions.

7.2. MATERIALS AND METHODS

7.2.1. Experimental design

For the present experiment, three plots, each with six trees, were allocated to the two watering treatments. Within each plot, two trees of similar height and diameter were selected for two defoliation treatments. At the start of this study in December 2007, the saplings were 12-months-old with an average height of 3.1 and 2.3 m and basal diameter at 0.15 m above the soil surface of 5.8 and 4.3 mm, in the irrigated and rain-fed treatments respectively.

For the present experiment, three plots of six trees were allocated to each of the two watering treatments. Within each plot, two trees of similar height and diameter were selected for two defoliation treatments. In mid-summer (2/01/2008; day 0),

approximately 75% of crown length was removed from one tree per plot, excluding apical foliage as far as the first fully-expanded leaf pair or approximately four leaf pairs. Leaves were snipped flush with the stem using long-nosed secateurs. The defoliation of the six trees took one day to complete. Approximately 60% of total leaf area, as estimated from the allometric relationships of O'Grady et al. (2006), was removed. The other six trees remained intact. The four treatments were described as follows: rain-fed undefoliated, rain-fed defoliated, irrigated undefoliated and irrigated defoliated.

The measurements of gas exchange, stomatal conductance and water potential were made on three fully-expanded leaves in the outer part of the canopy randomly selected. Leaves used for the measurements were all exposed to direct sunlight at the time of the measurement. Each set of measurements was made on the same day but on a different set of three leaves. Measurements were made two weeks before defoliation, 18/12/2007, and then on three occasions in summer; on 3/01/2008 on 24/01/2008 and 18/02/2008 or one, 22 and 47 days after defoliation.

7.2.2. Stem growth response

Height from the ground to the apical meristem (h) and diameter over-bark at 0.15 m above ground (d) of each tree were measured monthly from early summer 2007 (Dec 07) to mid-autumn 2008 (May 08). Callipers were used to measure the diameter over-bark. Mean height and diameter of all trees at the start of the experiment were 2.72 m and 50.5 mm, respectively. There were no significant differences in the mean h and d of non-defoliated (2.83 m, 52.2 mm) and defoliated (2.63 m, 48.8 mm) trees at the time of defoliation.

7.2.3. Gas exchange and diurnal measurement of leaf stomatal conductance

Light-saturated photosynthesis (A_{\max}) and stomatal conductance (g_s) were measured with an open flow infra-red gas analyser (CIRAS-1, PP Systems, Herts, UK). On each

tree, measurements were made in the two crown zones between 1000 and 1400 h AEST. Leaves were enclosed in a 250 mm² leaf chamber fitted with a light source providing a photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The ambient CO₂ concentration was maintained at 360 ppm. Leaf temperature varied between 16 and 21°C, and the readings were taken when g_s had stabilised.

Diurnal measurements of stomatal conductance (g_s) were made on the abaxial surface of leaves from the lower crown zone only using a steady-state porometer (Li-Cor 1600, Licor Inc., Lincoln, Neb.) using a circular 200 mm² broadleaf aperture cap. The pre-set humidity level during measurement was close to but just below ambient humidity. Measurements were made between 0800 and 1930 AEST on each of the four measuring days. Measurements were made at 60–90 min intervals. Leaf g_s was converted from cm s⁻¹ to mol m⁻² s⁻¹.

7.2.4. Leaf water potential

Diurnal patterns of leaf water potential (Ψ) were measured using a Scholander-type pressure chamber. For each measurement, three leaves were excised and placed into plastic bags within an insulated ice-cooled container, where they remained until measurement, usually within 10 min of excision. Pre-dawn leaf water potential (Ψ_{pd}) was used as an estimate of soil water potential. Diurnal measurements were done between 0430 and 2230 AEST, at 60–90 min intervals, on the same measuring days as gas exchange and stomatal conductance. All leaves were collected from the lower crown zone.

7.2.5. Whole tree water use

Sap flow (Q) was measured at 1.3 m height using SF100 sap flow probes and loggers (Greenspan Analytical, Warwick, Queensland, Australia). Description of the material used to measure tree water use is given in chapter 6. Water use was expressed on a tree

basis as E (leaf area) by dividing by leaf area. Leaf area of each tree was calculated using allometric equations developed for trees at this site (O'Grady et al. 2006). Sap flow measurements were conducted between 18/12/2007 and 24/02/2008. Canopy conductance (G_C) was calculated from transpiration per (E) following Ewers et al. (2005) (see Chapter 6). Plant hydraulic conductance (K_P) was calculated from transpiration (E) and difference in water potential ($\Psi_{pd} - \Psi_{md}$) (Loustau and Granier 1993) (see Chapter 6).

7.2.6. Data analysis

repeated measures ANOVA for was used to evaluate the effects of treatments on all parameters using the statistical package Genstat10 (GENSTAT Committee 1989). When multiple comparisons were made, the least significant difference (LSD) t-test was used. A group regression procedure (McPherson 1990) was used to determine the effect of defoliation treatment on the slope and intercept of the relationship between A_{max} and g_s . This procedure tests the hypotheses that (1) the regression lines have common slope allowing for the possibility that they have different intercepts, and (2) that the same line applies to all defoliation treatments. Linear regressions were fitted to data relating average values of A_{max} and hydraulic conductance. All regressions were performed using Genstat 10.

7.2.7. Modelling analysis with CABALA

The effect of defoliation on productivity of *E. globulus* was modelled using CABALA version 2.1. The parameterisation of CABALA for *E. globulus* and the source of parameter values are provided in Battaglia et al. (2004). The model was run for the experiment two at Pittwater (see description in Chapters 2 and below). Site-specific site (climate, soils) and regime (seedling size and leaf area, planting date, fertilising regime, harvest date) files were developed for the site (see Chapter 2) and are outlined in Table 7.1. At the start of simulations seedlings were 100 mm tall, had a leaf area per seedling

of 0.15 m² and foliar nitrogen concentration was 2.5 g.g⁻¹. Meteorological data from nearest weather station (Hobart airport; 47°50'S, 147°30'E) over the modelling period were downloaded from the Metservice climate base.

Within CABALA, defoliation events are specified for three equal horizontal (lower, middle, upper) and two vertical crown zones (inner and outer). The defoliation regime simulated top-down defoliation of 60% of leaf area, meaning that leaf area was lost from the upper and mid crown zones only, and from both inner and outer crown positions.

The model runs on a daily time-step with carbon and water fluxes calculated as the average of mid-morning and midafternoon conditions (Sands 1995). Tree water stress is determined by the average pre-dawn water potential of the soil volume occupied by the tree roots. Comparisons of observed (n=12) and predicted growth were made primarily using stand basal area, CABALA's conventional output variable that is most closely related through mean tree diameter at breast height (mm) and stand tree volume (m³ ha⁻¹) to the allometric (power) relationships within CABALA that are used to distribute assimilated carbon (and therefore biomass) amongst tree components. Assumptions were made when developing a process-based model and complex interactions can be expected that could threaten the model sensitivity. This problem accounts for the deliberate choice of using applicable parts of existing models that have already been tested for their sensitivity and consistency (Pinkard et al. 2009).

To simulate the likely impacts of pest attack on plantation productivity, as defined by the volume involved an appraisal of the impacts of 50% defoliation at specific sites, to provide an indication of the spatial variability in productivity responses of *E. globulus* to insect defoliation. The climate data for that year was then looped so that a full rotation could be run with CABALA (10 years). Defoliation event was applied repeatedly over the 10 years at the same time period and the same level.

Table 7.1. Detailed CABALA site inputs for Pittwater Experimental research Station, southern Tasmania.

Inputs details	Site
Site latitude (°)	-43
CO ₂ atmospheric (ppm)	350
Susceptibility to water logging (yes/no)	No
Freshwater table depth (cm)	200
Initial available soil water (mm)	Full
Soil texture	Sand
Thickness of soil horizon profiles: upper, middle, lower (cm)	175, 30, 0
Proportion of layers upper, middle, lower horizon profile comprising stones (%)	0
Water-holding capacity of layers 1, 2, 3 (mm)	60, 85, 0
Thickness of user defined layer 1, 2, 3 (cm)	10, 10, 30
NH ₄ content of 1, 2, 3 soil layers (kg ha ⁻¹)	6
C:N ratio of 1, 2, 3 soil layers	40
Organic matter content of 1, 2, 3 soil layers (proportion)	0.7, 0.2, 0.1
Bulk density of 1, 2, 3 soil layers (g cm ⁻³)	1.3
pH of 1, 2, 3 soil layers soil layers	7
Within-row tree spacing (m)	3
Row spacing (m)	3

7.3. RESULTS

7.3.1. Compensatory responses

Over the period of the experiment, there was significant effect of the watering treatment on maximum photosynthetic rate (A_{\max}) and stomatal conductance (g_s) ($P < 0.1$), and A_{\max} and g_s of trees in the irrigated treatment were always higher ($14.77 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $283.4 \text{ mmol m}^{-2} \text{s}^{-1}$, respectively) than those in the rain-fed treatment ($11.62 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $175.1 \text{ mmol m}^{-2} \text{s}^{-1}$, respectively). Defoliation treatment had no significant effect on A_{\max} and g_s of rain-fed defoliated trees compared with rain-fed undefoliated trees ($P > 0.1$; Figure 7.1a and 7.1b). One day following the defoliation, A_{\max} of irrigated defoliated trees decreased significantly compared to that of irrigated undefoliated trees ($P < 0.05$; Figure 7.1a). However, 22 days after the defoliation, significant increases in A_{\max} and g_s were measured in irrigated defoliated trees compared with irrigated undefoliated trees ($P < 0.1$; Figure 7.1a and 7.1b). A_{\max} and g_s of irrigated defoliated trees were 26 and 72% higher, respectively, than those of irrigated undefoliated trees.

7.3.2. Regulation of tree's capability for transporting water to the leaves

Pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials varied significantly by date, and watering treatments ($P < 0.1$). Predawn and midday leaf water potential were higher for trees in the irrigated treatment than those in the rain-fed treatment. Before the application of defoliation treatment, early summer, the watering treatments had no significant effect on diurnal Ψ ($P > 0.1$). Following the application of the defoliation treatment, defoliation did not affect significantly Ψ for any measurement day ($P > 0.1$). However, trees in the defoliated treatment had higher Ψ than trees in the undefoliated treatment (Figure 7.2).

Trees in all treatments exhibited similar daily variations in E_{\max} and G_{Cmax} (Figure 7.3). In general, E_{\max} and G_{Cmax} were higher in the irrigated than rain-fed treatments irrespectively of the defoliation treatment, although differences were not significant ($P > 0.1$; Figure 7.3). Defoliation treatment had a significant effect on E_{\max} and G_{Cmax} ;

they were higher in defoliated than in undefoliated trees, irrespectively of the watering treatment ($P < 0.1$; Figure 7.3). This increased response to defoliation was observed between 04/01/08 and 01/02/08, and reached its maximum around the 06/01/08 for E_{\max} and around the 24/01/08 for G_{\max} .

Soil-to-leaf hydraulic conductance (K_P) was not significantly affected by watering and defoliation treatments ($P > 0.1$), although strong trends were apparent in the data. Over the period of the experiment, irrigated defoliated trees exhibited the highest mean K_P ($18.6 \pm 4.6 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$), rain-fed undefoliated trees the lowest ($12.2 \pm 2.2 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$). Rain-fed defoliated and irrigated undefoliated trees had similar mean K_P ($13.5 \pm 1.6 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$). One day after defoliation, K_P was lower in rain-fed defoliated and irrigated defoliated trees than rain-fed undefoliated and irrigated undefoliated trees ($P > 0.1$; Table 7.2). On 24/01/2008, K_P was higher in rain-fed defoliated and irrigated defoliated trees than rain-fed undefoliated and irrigated undefoliated trees ($P > 0.1$; Table 7.2). Across all treatments, there were significant positive correlations between K_P and A_{\max} (Figure 7.4a). This relationship was stronger when K_P was compared with upper canopy A_{\max} only (Figure 7.4b).

7.2.3. Defoliation offset the negative effects imposed by the rain-fed treatment on growth

Diameter increment (d_{inc}) of irrigated trees was 34% higher ($P < 0.1$), and height increment (h_{inc}) was 26% greater ($P > 0.1$) than these of rain-fed trees after 13 months of watering treatment. Application of defoliation treatment had no significant effect on d_{inc} ($P > 0.1$; Figure 7.5a). Defoliation treatment significantly affected h_{inc} in both watering treatments ($P < 0.1$; Figure 7.5b), but the response differed between watering treatment. Height increment of irrigated defoliated was 31% less than that of irrigated undefoliated trees, whereas h_{inc} of rain-fed defoliated trees was 75% higher than that of rain-fed undefoliated trees.

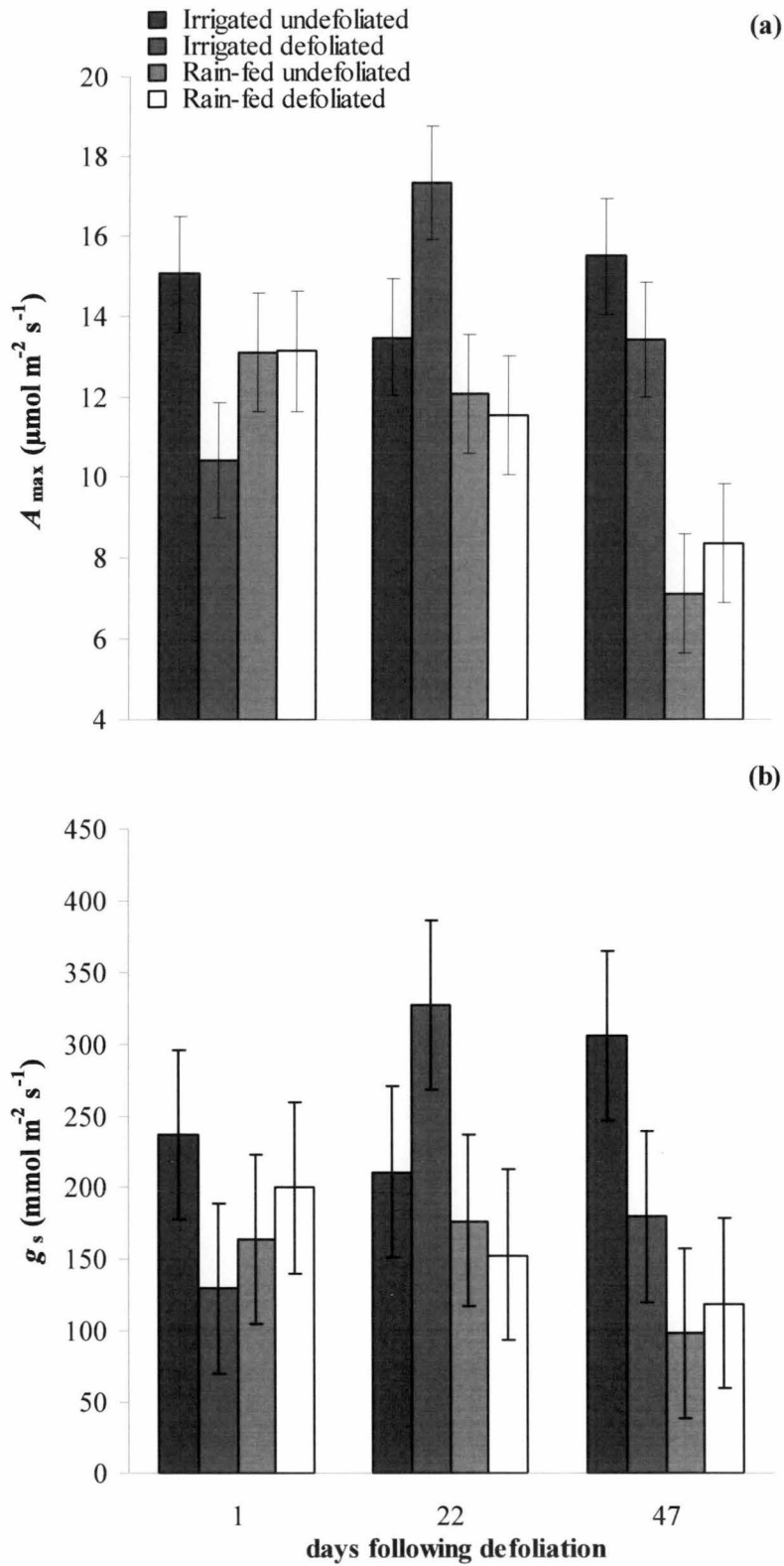


Figure 7.1. Means of (a) maximum photosynthesis (A_{\max}), and (b) leaf conductance (g_s) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees growing in Southern Tasmania. Errors bars show least square differences ($\alpha=0.05$).

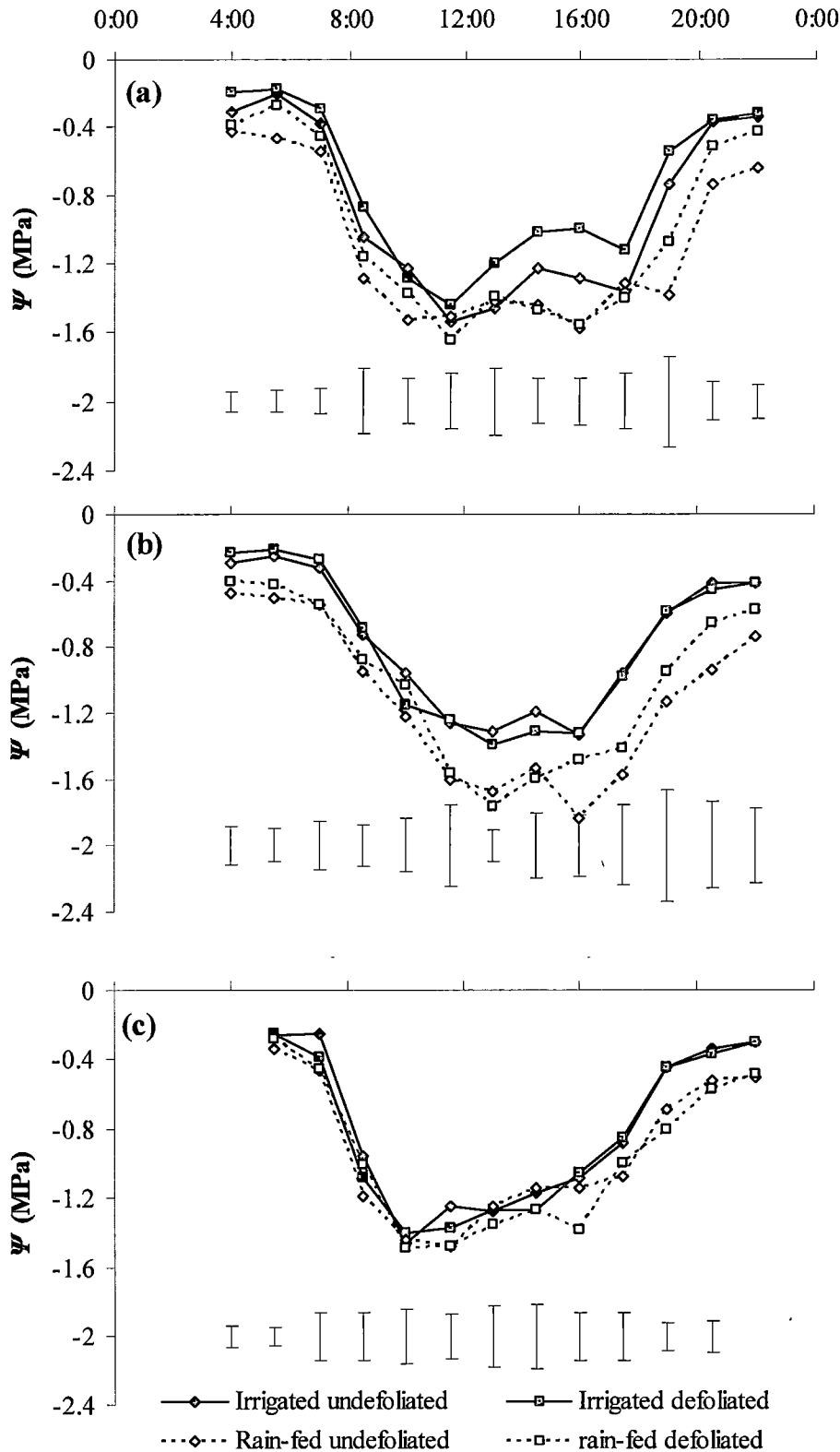


Figure 7.2. Diurnal patterns of leaf water potential (Ψ) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees in (a) 03/01/2008, (b) 24/01/2008 and (c) 18/02/2008. Data represent the mean and standard error of measurement on three trees in each treatment during summer 2007/2008. To clarify the error bars are only represent at the bottom ($\alpha=0.05$).

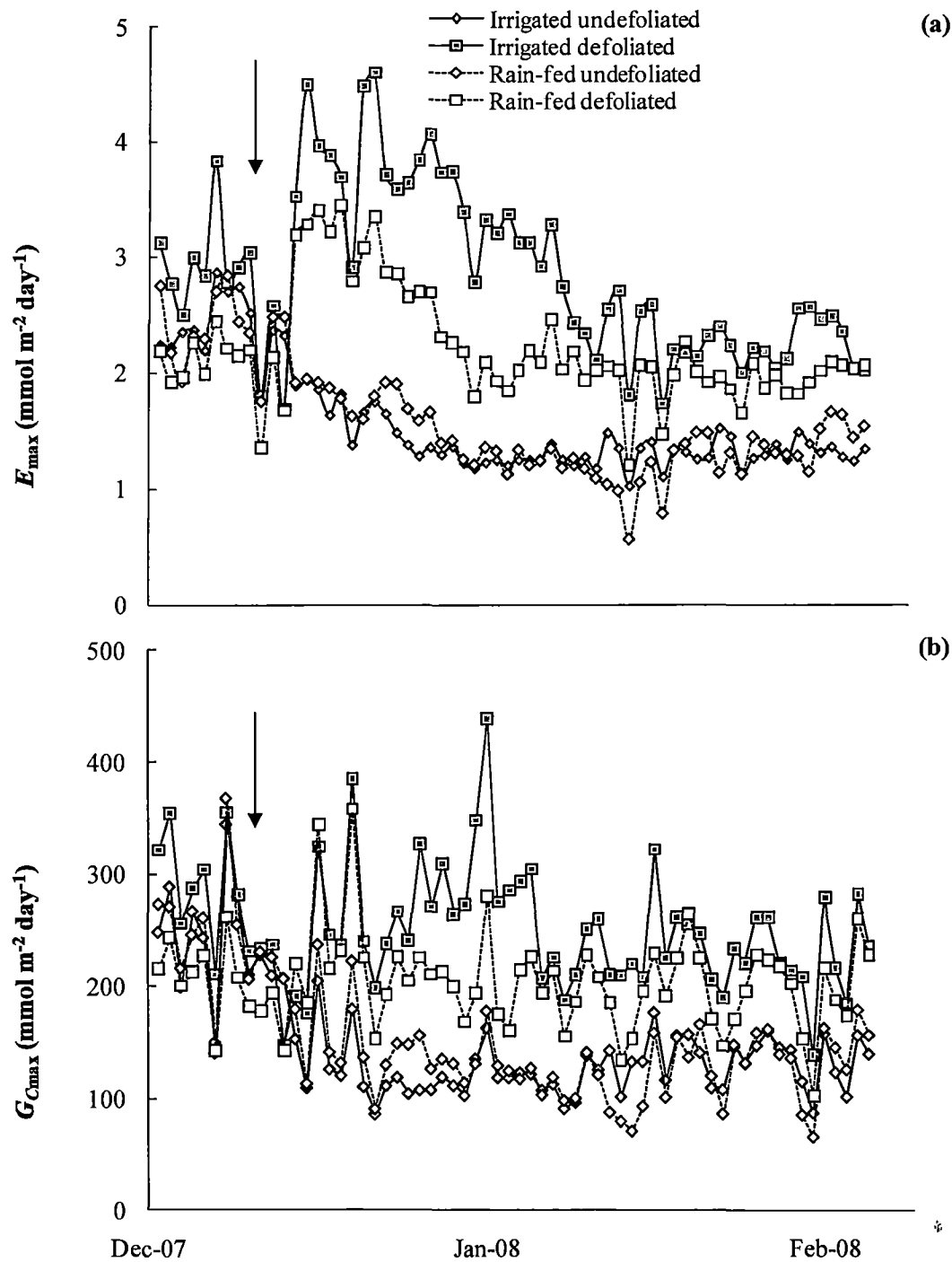


Figure 7.3. Daily variation of (a) maximum transpiration rates (E_{\max}) and (b) maximum canopy conductance ($G_{C\max}$) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* at the Pittwater research plantation in southeast Tasmania over the growing season of 2007/2008. Arrows represent the time of the defoliation occurred.

Table 7.2. Mean \pm SE plant hydraulic conductance (K_P) of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees in plots growing under similar atmospheric conditions. Measurements were made on three occasions after the defoliation.

<i>Days</i>	K_P (mmol m ⁻² s ⁻¹ MPa ⁻¹)			
	<i>Rain-fed undefoliated</i>	<i>Rain-fed defoliated</i>	<i>Irrigated undefoliated</i>	<i>Irrigated defoliated</i>
3/01/2008	19.54 \pm 3.89	11.55 \pm 4.60	17.22 \pm 3.55	12.07 \pm 4.17
24/01/2008	10.91 \pm 0.10	18.33 \pm 2.38	11.82 \pm 6.55	26.00 \pm 12.36
18/02/2008	7.49 \pm 1.64	12.45 \pm 2.87	11.85 \pm 4.24	18.35 \pm 6.69

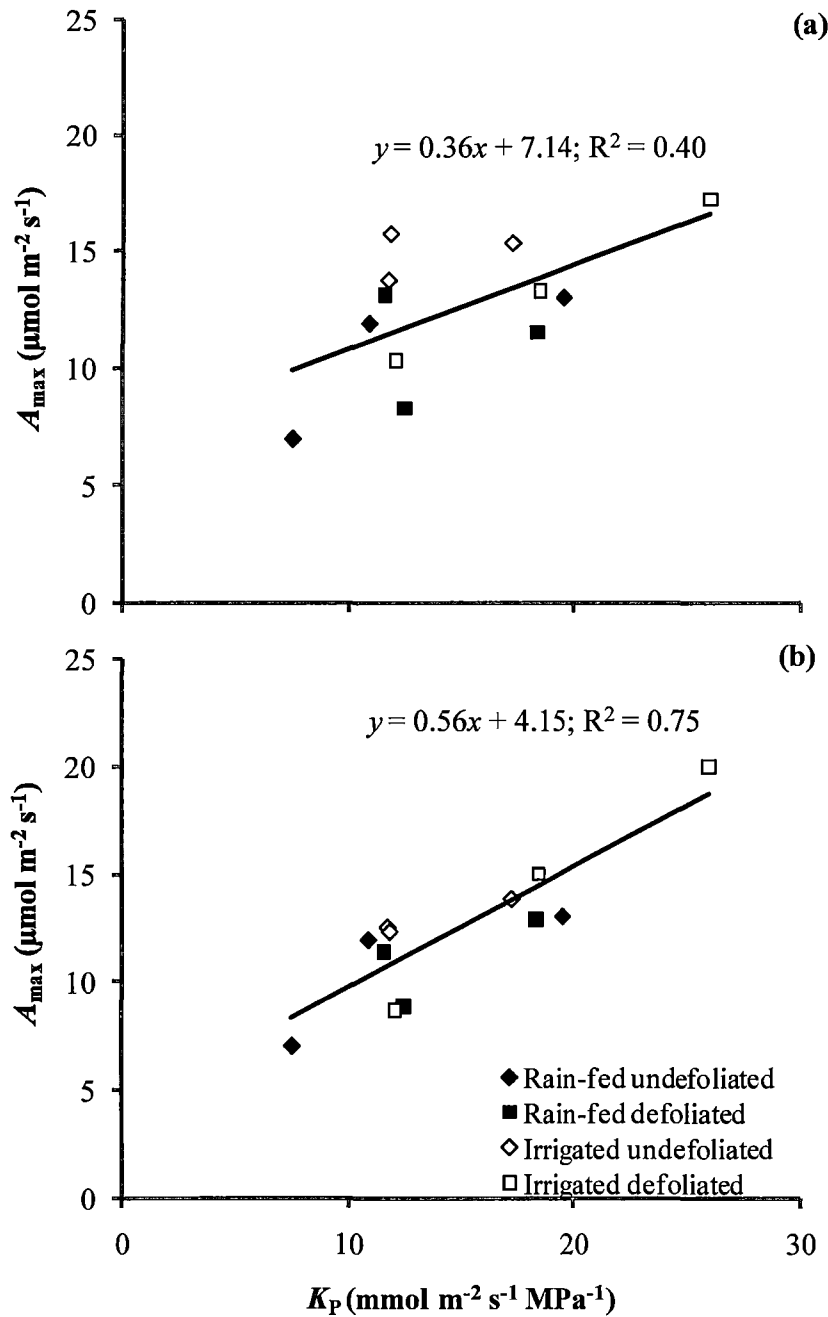


Figure 7.4. A significant linear correlation between mean soil-to-leaf hydraulic conductance and (a) canopy averaged maximum photosynthesis (A_{max}), and (b) mean A_{max} from the upper crown in a sample of irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus*. Values are presented for all measuring dates. Pooled data are presented by the solid line.

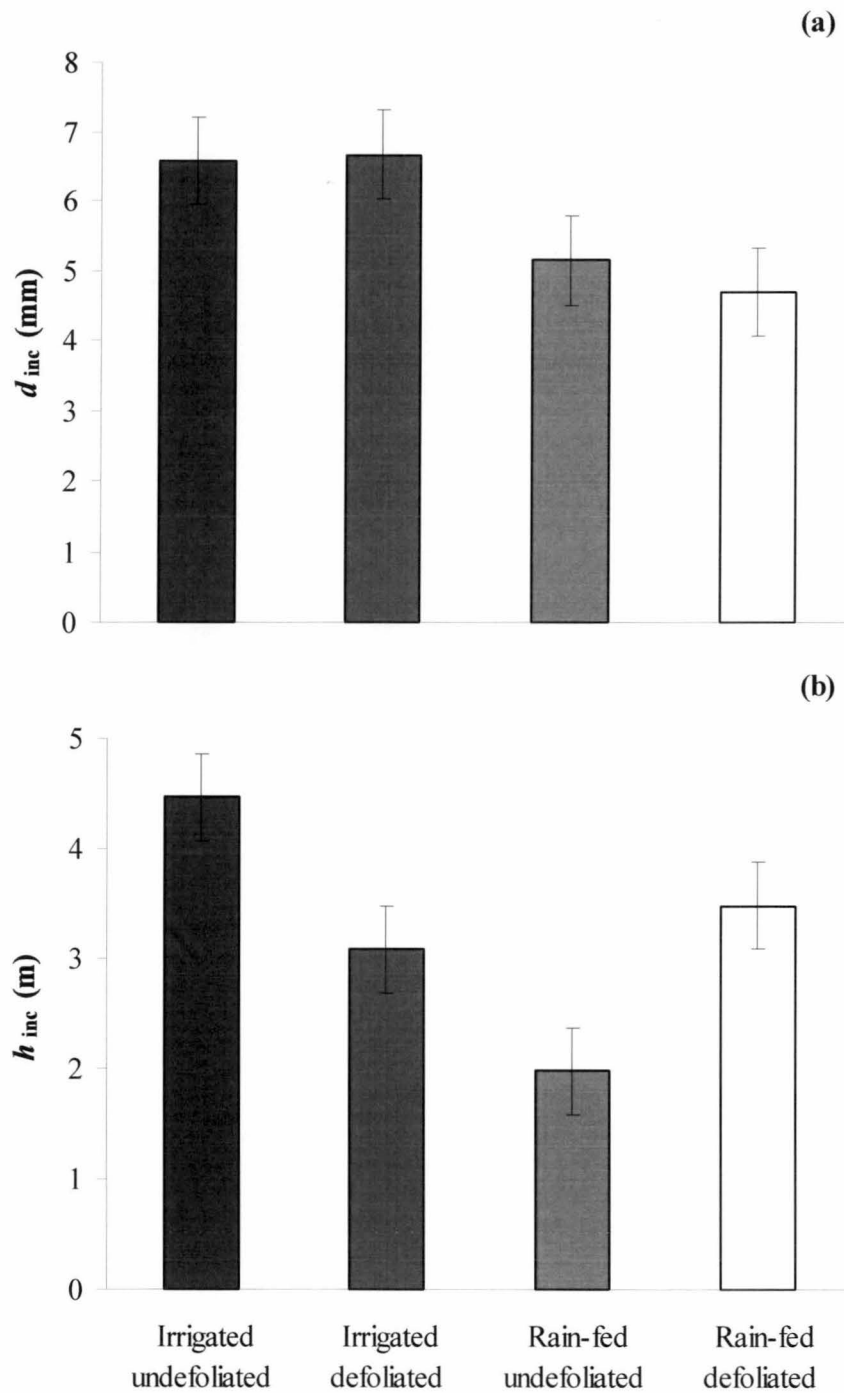


Figure 7.5. Mean increments of (a) height and (b) over-bark diameter for irrigated undefoliated, irrigated defoliated, rain-fed undefoliated, rain-fed defoliated *E. globulus* trees. Errors bars show standard error differences ($\alpha=0.05$).

7.3.4. How well does CABALA predict impacts of defoliation on tree response?

For both water regimes, CABALA generally gave good predictions of tree diameter and volume (Figure 7.6), and relationships between predicted and observed diameter for all pooled data were also very strong with R^2 above 0.9. At the end of the experiment, the predicted diameter was 20% lower than the observed value across all treatments (Figure 7.6a). Measured values of volume across all treatments at the end differed from modelled values by no more than 6%, except in rain-fed defoliated treatment (Figures 7.6b). In this instance, the predicted volume was 23% lower than the observed value. The predictions give us general confidence in the model for examining the effects of partial defoliation on volume of *E. globulus* under low and high watering condition. Figure 7.7 gives an example of rotation-length growth responses for *E. globulus* growing under current climate conditions. In general, reductions in stem volume associated with defoliation occurred soon after defoliation, and were then sustained until harvest age. After 10 years, repeated defoliation event reduced tree productivity by 10%, and this independently of the watering condition. However, the irrigation treatment offset the effect of the defoliation by 8% compared to rain-fed treatment (Figure 7.7).

7.4. DISCUSSION

Defoliation is associated with many morphological and physiological responses (Prins and Verkaar 1992; Trumble et al. 1993; see previous chapters). A common response is an increase in the light-saturated photosynthetic rate (A_{max}) and stomatal conductance (g_s) of residual and new leaves compared to those of non-defoliated controls (Heichel and Turner 1983; Reich et al. 1993; Trumble et al. 1993; Vanderklein and Reich 1999; Pinkard et al. 2007; Turnbull et al. 2007). It is well established that A_{max} and g_s decrease in response to reduced water availability (Epron and Dreyer 1990; Eamus et al. 1999). In this study, photosynthetic responses to defoliation were variable and substantially modified by water availability.

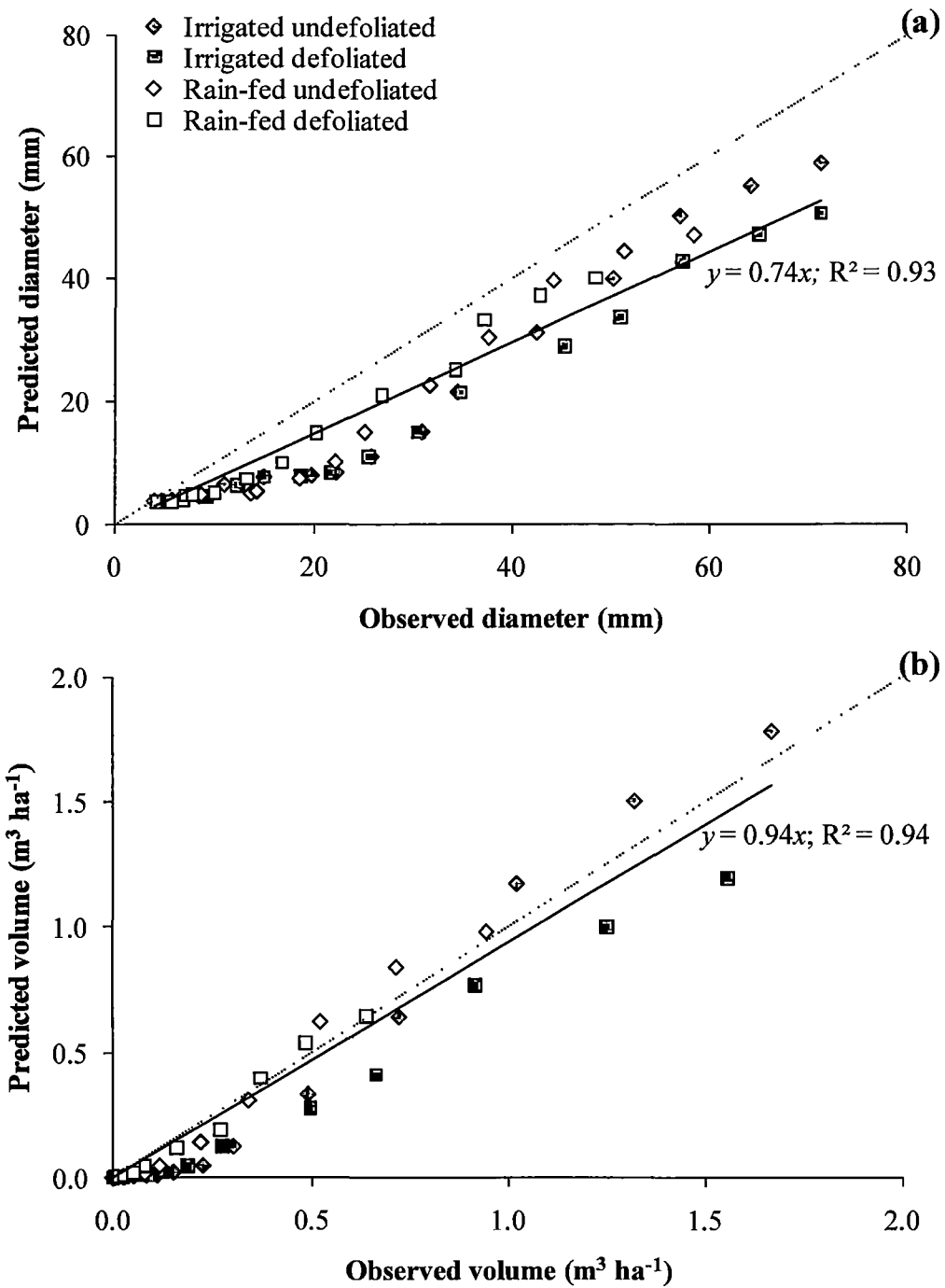


Figure 7.6. Measured versus predicted using CABALA (a) diameter at breast height (b) and (b) stem volume of rain-fed undefoliated, rain-fed defoliated, irrigated undefoliated, and irrigated defoliated *E. globulus* grown in trial plantation in Southern Tasmania. Seedlings were considered to be 0.1 m tall with 0.015 m^2 of leaf area. Solid trendline represents the linear regression for all data put together ($P < 0.001$). On each plot the dotted line represents the 1:1 line.



Figure 7.7. Predicted entire stem volume of *E. globulus* growing in southern Australia, from planting to harvest at age 10 years. Values are presented for rain-fed undefoliated, rain-fed defoliated, irrigated undefoliated, and irrigated defoliated. Defoliation occurred each year at the same time period and at the same level.

Irrigation led to a transient up-regulation of A_{\max} following defoliation that could be attributed to increases in g_s and leaf hydraulic conductance. The commonly observed up-regulation of photosynthesis and compensatory growth in response to defoliation can thus be linked to a plausible mechanism, at least in irrigated trees. Previous studies of defoliation on the growth of young *E. globulus* trees indicate reduced diameter and height growth (Pinkard et al. 2006a; Pinkard et al. 2007), or no significant change in diameter following a short-term stimulation of growth (Eyles et al. 2009b). In this study, height growth was reduced in irrigated defoliated *E. globulus* but diameter growth was increased five months after defoliation. Low water availability under rain-fed conditions did not constrain this expression of tolerance to defoliation. Thus growth of trees in the defoliated treatment was unaffected five months after defoliation, in spite of removal of 60% of total tree leaf area from the upper crown. These findings are consistent with those of Eyles et al. (2009), who demonstrated that the stem growth response to partial defoliation may be robust even under low water supply.

In contrast, there was no evidence of increased A_{\max} or g_s in residual foliage of rain-fed defoliated trees, despite observations at the whole canopy scale of increased canopy conductance, G_C . *E. globulus* is sensitive to water deficits, and gas exchange is known to be limited at $\Psi_{pd} < -0.5$ MPa (White et al. 1999; O'Grady et al. 2008), just below the levels recorded in this study. Another possible reason for the disparity between leaf- and canopy-level conductances in rain-fed treatments is that the sampling was logistically constrained to a limited number of trees in each treatment. In this study, leaf-level measures of g_s were an instantaneous representation of ambient conditions whereas G_C was calculated from sap flux measurements and thus averaged whole-canopy behaviour. The results may demonstrate that water status must be considered when assessing the impact of defoliation on plants.

When soil water is limiting, removal of the transpiring surface area *via* defoliation of the plant canopy may act to reduce the demand for water and conserve soil moisture (McNaughton 1983; Brown 1995). This argument is used to explain the often observed increase in leaf water potential, transpiration rate and canopy conductance following defoliation (Stephens et al. 1972; Welker and Menke 1990; Reich et al.

1993; Vanderklein and Reich 2000; Gieger and Thomas 2002). In this study, leaf water potential, Ψ of the defoliated and defoliated-irrigated trees was higher, albeit not significantly, than control and irrigated trees. Increases in maximum E and G_C , and K_p following defoliation were also observed. These responses imply an improvement in tree water transport following defoliation, irrespective of the watering treatment. Light-saturated photosynthesis, A_{\max} in these trees was linearly related to K_p and these observations are consistent with isohydric regulation of plant water status (Meinzer 2002, Mencuccini 2002). *E. globulus* trees have demonstrated strong isohydric control of plant water status (O’Grady et al. 2008). O’Grady et al (2008) observed a linear decrease in the difference between minimum (predawn) and maximum (midday) leaf water potential ($\Delta\Psi_{\text{pd-md}}$) under progressive soil water deficits and argued that this was indicative of isohydric regulation of gas exchange. Isohydric regulation of plant water status through strong stomatal control of transpiration is advantageous in woody plants as it prevents water potentials falling to dangerous levels and risking serious xylem dysfunction, where the cost of recovering hydraulic function may be high (Franks et al. 2007).

If A_{\max} had been limited by water supply, the increased Ψ_{pd} of the residual foliage after partial defoliation may be the primary signal for the often observed up-regulation photosynthetic activity. In this study, A_{\max} and g_s were primarily regulated by the tree’s capacity to transport water to the leaves during periods of water stress exerted by low water supply and/or defoliation. Soil-to-leaf hydraulic conductance, K_p explained a considerable amount of the variability in the observed A_{\max} and g_s among water availability and defoliation treatments. Strong correlations between the hydraulic conductance of branches and leaves and photosynthetic capacity have been observed in a number of studies (Hubbard et al. 1999; Brodribb and Feild 2000; Sperry 2000; Hubbard et al. 2001; Meinzer 2002; Brodribb and Holbrook 2003). Defoliation alleviates water stress by reducing hydraulic limitations; hence more water is available to the remaining foliage. The correlation between K_p and A_{\max} or g_s illustrates the importance of plant hydraulic characteristics in co-ordinating carbon uptake through the stomata while regulating water loss, due to strong selection for hydraulic–stomatal co-ordination within the leaf as well as throughout the whole plant (Brodribb et al. 2004). Partial defoliation in young *E. globulus* appeared to have reversed the

physiological effects of low water supply, possibly *via* a hydraulic signal resulting in stomatal adjustment that minimises fluctuations in Ψ (Meinzer and Grantz 1990).

The results in this experiment are consistent with a simple hydraulic model that predicts how G_C at a given value of D should vary with K_P , sapwood-to-leaf area ratio (A_S/A_L) and the water potential gradient between the soil and the leaf ($\Delta\Psi_{pd-md}$) (Whitehead and Jarvis 1981; Ewers et al. 2005):

$$G_C = K_P \cdot \frac{A_S}{A_L} \cdot \frac{1}{D} \cdot (\Delta\Psi_{pd-md} - h\eta) \quad \text{Eq. 7.2}$$

where η is the viscosity of water at 20°C and h is the tree height. Isohydic plants maintain reasonably constant minimum leaf water potential. Partial defoliation increases the sapwood-to-leaf area ratio which, for a given D , could result in either an increase in G_C , K_P or both as observed in this study. Previous defoliation experiments (Meinzer and Grantz 1990; Pataki et al. 1998) have shown similarly that stomata can respond rapidly to defoliation and it was argued that this response was related to the associated increase in K_P . Our findings appear to support in part the conceptual hydrological model where defoliation results in increased A_S/A_L and K_P , and constant $\Delta\Psi_{pd-md}$, resulting in increased G_C , and suggest that G_C of defoliated trees was directly related to hydraulic adjustment.

Rain-fed defoliated *E. globulus* was able to alleviate the effect of low water supply on growth and also tolerate the simulated herbivory *via* an improvement in water status. This could have a potential ecological importance for eucalypt plantations growing in drying soils. A reduction in water stress resulting from defoliation may improve tree survival to dry period. In natural stands and plantations, prolonged periods of low water availability occur regularly, although with differing frequency and intensity. Under the current climate predictions forest would be often more prone to drought events (IPCC 2001). Defoliation by herbivores does not usually occur as a single event but repeatedly over several years at varying levels of intensity (Straw et al. 2000). This raises the following questions. How would growth respond to repeated drought and defoliation? Would repeated defoliation act to reduce the long term effect of

drought? From these results, I cannot extrapolate that improved plant water status by defoliated trees versus non-defoliated trees over consecutive years would provide an enduring benefit. This study emphasizes the need to consider the whole plant when assessing plant physiological responses to stressors (Meinzer 2003). It is clearly of benefit for comparative studies of plant performance to include simultaneous measurements over a range of scales.

Of the 1.8 million ha of plantations in Australia, approximately 42% are hardwood, and of those most are eucalypts (Anon., 2005). Much of the plantation estate is sited in areas with rainfall ranging from 400 mm upwards (Anon., 2005), and new plantation development increasingly is occurring in low rainfall environments. Lower rainfall environments are likely to become more favourable for insect attack under changing climate (Rosenzweig et al. 2001; Pinkard et al. 2009a), meaning that defoliation severity may increase at such sites. Our results from the modelling analysis suggest that the effects of defoliation due to pests on plantation productivity should not be ignored when considering future management of existing or planned forest plantations. However, irrigation application may offset the effect of insect defoliation on tree productivity. The approach developed here with CABALA may provide plantation managers with a tool to appraise risk and examine the possible impacts of management interventions designed to reduce the impact of pest attack on plantation productivity. It is an example of the possible effects of pest attack on plantation productivity, which could be extended in the future to include other defoliating pests.

Chapter 8: Implications for management.

Insects can damage trees at every stage of their development. However, most trees are host to a range of insects throughout their life and may cope well with minor insect damage. In Tasmania, the cost of lost production from unmanaged defoliation is significant, about \$150,000 per annum for the past two years (Forestry Tasmania, pers. comm.). Control of pests is essentially centred around the use chemical insecticides, providing effective control, but it was not without with environmental and economic constraints and costs (\$40 to 55/ha; Forestry Tasmania, pers. comm.). Nowadays, the ultimate objective of Forestry Tasmania in pest control is to establish plantations that require no spraying with insecticides throughout their rotation. On the contrary, insecticides are still widely applied elsewhere. Aerial spraying of insecticides on large surface of *E. globulus* in Western Australia is the only available technology that offers a viable means of controlling pest insects in plantations (Francisco Tovar, pers. Comm.). However, forest industry along with farmers recognises the shortcomings of this technology, and is actively researching alternative technologies. Ecologist and entomologist are becoming involved in the development of an ecologically compatible methodology of pest control known as: integrated pest management (IPM).

Knowledge of the forecasted damage level aids the decision-making process in an era of greater accountability for implementation of pest control options and the need to be able to forecast yield of timber to manage forests on a sustainable basis. Hence, a multidisciplinary approach is required for successful application of IPM programs. Research is being conducted currently in order to achieve this with studies focusing on options such as the identification of resistant species/provenances (Farrow et al. 1994; Floyd et al. 2002; Jones et al. 2002), selection of resistant genotypes (Jones et al. 2002; Rapley et al. 2004), developing biological control such as the use of trap trees or pheromones (Steinbauer et al. 2006; Collett 2001), or parasitism (Loch 2008). However, to better understand the regulation and the impacts of these pest control

options, there is a need for deeper expertise in physiological processes of host plant. The usefulness of understanding physiological responses to silvicultural practices has been demonstrated with the development of site-specific pruning and thinning prescription for *E. nitens* stands (Pinkard 1997; Medhurst 2000).

A major problem encountered in this thesis was the lack of statistical significance of the results. This lack of statistical significance was likely to be attributed to the small size of the samples, which was imposed by logistical constraints of the study such as: working with 13-meter trees, access to the plantation, safe access to the upper canopy, use of heavy machinery. The experiments carried out in the thesis were designed to fit within these constraints and as a consequence limited the number of trees. Statistical power analysis has been advocated and sometimes used to improve research designs and to facilitate interpretation of statistical results in the applied sciences (Steidl, 1997; Martínez-Abraín, 2008). The technique of statistical power analysis aims to decide how large a sample is needed to enable statistical judgments that are accurate and reliable. In the context of the study on tree growth and biomass, the number of trees needed to perform a reliable ANOVA procedure at 50% and 80% would have been $n=9$ and $n=17$, respectively (Figure 8.1). Addressing complex ecological research questions often requires complex empirical experiments. However, due to the logistic constraints of empirical studies there is a trade-off between the complexity of experimental designs and sample size. In an effort to encourage more accurate analysis of response data, I addressed the statistical treatment of physiological response obtained by measuring the response of each experimental unit to all the investigated levels of the environmental factor governing the response to insect defoliation. Such an experimental design, typical in eco-physiological research, refers to as a repeated-measure design in the statistical literature. Repeated-measure designs have the advantage of increasing the precision of the treatment analysis by accounting for inter-individual variation (D. Ratkowsky, personal communication).

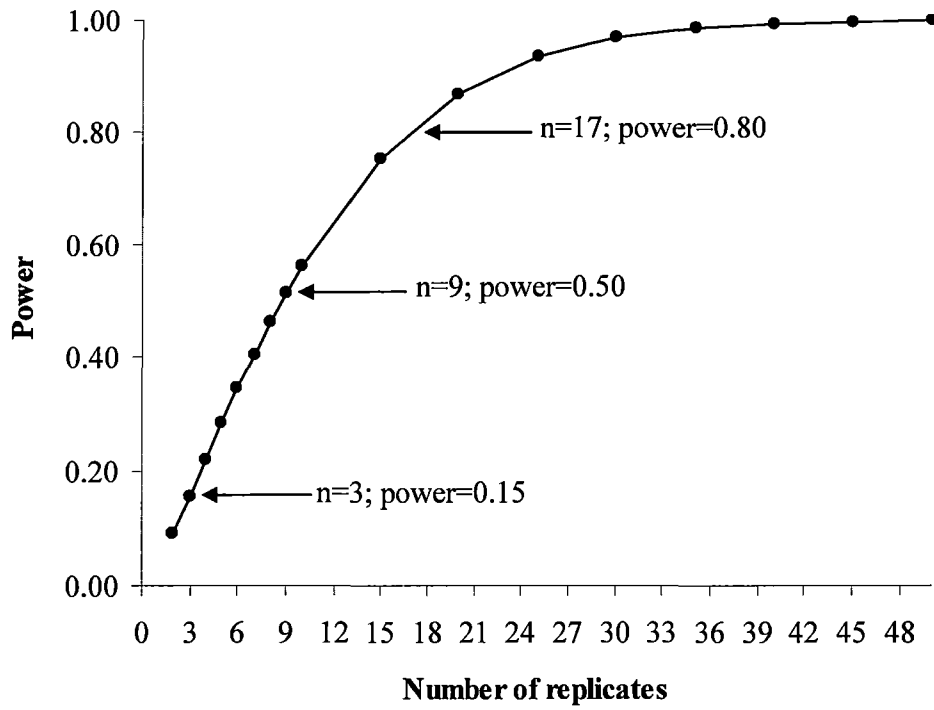


Figure 8.1. Results of a power analysis to detect difference in growth between undefoliated and defoliated, based on a one-way ANOVA analysis, and $\alpha = 0.05$ (Lenth, 2009).

In combination with the pot experiment, it was possible to develop an understanding of the physiological responses to partial defoliation in determining the impact on growth, and the importance of integrating site conditions in driving the response. The experiments that formed the basis of this thesis were conducted in a plantation using artificial defoliation. Artificial damage is a popular method in plant-insect experiments, because it offers several practical convenience and benefit over the use of real herbivores (Hjältén 2004). Progress in understanding plant physiological responses to insect defoliation resulted primarily from a consideration of injury types. Based on the primary physiological parameters evaluated in the Chapter 3, artificial defoliation can only mimic the nature and degree of defoliation caused by leaf chewing insects. But the following question arises when it comes to interpret the results from the field experiments where artificial defoliation was used: what would have happened with real herbivores? This study demonstrated that artificial and insect defoliations both displayed increased photosynthetic rates, and similar responses were then reported in the field experiments. In contrast, the contrasting results reported in the growth response may mislead on the interpretation of the results. Developing a better understanding of the effect of bud removal on apical dominance may improve our interpretation and extrapolation of the results when it comes to real herbivores. Therefore I am confident that the use of artificial defoliation in the field experiments produced a reasonable approximation of natural defoliation on the growth response and physiological processes at time of defoliation and during the recovery period. Understanding plant physiological responses to herbivory and tracking the impact of those responses on tree productivity can provide a quantitative and systematic method for evaluating loss of growth. Increased gas exchange associated with defoliation was observed in young and mature trees, although this response was only transient (Chapters 3, 5 and 7). This response was also related to improvement in plant hydraulic status (Chapters 6 and 7), which in turn is consistent with the hydraulic model. These mechanisms have likely been responsible for the reduction of defoliation effects on growth.

Increases in the occurrence of drought conditions and temperatures outside the optimal range for growth are possible under future climate projections (Hughes et al. 1996;

Hughes 2003), and are prone to reduce plant productivity and likely to modify the ability of trees to respond insect defoliation (Ayres 1993). A longstanding concern regarding characterising stress response plants is the potential for interactions among stressors. Indeed, the ability of abiotic stressors to interact with biotic stressors is well known (Jones and Coleman 1991; Waring 1991). Many *E. globulus* stands are now planted into sites where periodic water limitation occurs. In dry environment, the productivity of *E. globulus* is strongly limited by moisture stress (Osório et al. 1998). However, there is a very limited understanding on how defoliation and water limitation availability influence the productivity trees over periods of several years; and yet process-based simulation model was used as a tool (Hogg 1999). Results from the present study indicate that physiological responses of *E. globulus* to defoliation may be dependent on water supply (Chapter 7). Over a short-term period, it was concluded that defoliated trees were able to alleviate the effect of water stress by improving tree's capability of transporting water to the leaves. Also, the findings were conforming to the theoretical hydraulic model. However, it cannot be assumed that defoliation under dry conditions will result in fewer impacts on wood production; it would be dependent on other parameters such as the frequency and intensity of defoliation, as well as the duration of the drought period.

The effects of defoliation on growth and physiology of *E. globulus* has been studied extensively (Collett and Neumann 2002; Pinkard 2003; Pinkard et al. 2006b; Pinkard et al. 2007; Turnbull et al. 2007; Eyles et al. 2009b). Briefly, reductions in growth were lower than the level of reduction in removal of leaf area (Collett and Neumann 2002; Pinkard 2003; Pinkard et al. 2006b), as *E. globulus* can respond to defoliation through compensatory responses, which include increased allocation to above-ground biomass (Eyles et al. 2009b) and increases in photosynthesis (Pinkard 2003; Pinkard et al. 2007; Turnbull et al. 2007). While these studies have generally involved short term effects at a time scale of a few weeks to up to 20 months; none has examined the rotation-length implications of defoliation and no long-term data are available; hence the long-term effects remain uncertain. Yet, management options are needed for long time scales; hence there is a necessity for using models. Growth models draw upon knowledge gained from physiological research conducted on many aspects of plant growth and prove to be a useful way of exploring uncertainty through testing different

hypotheses of system interactions over a long term period (Pinkard et al. 2009a; 2009b). Under changing climate, pest distribution, feeding activity and plant response would be highly affected (Wardlaw 1990; Sutherst and Floyd 1998; Patterson et al. 1999; Bale et al. 2002). Likely, these could create substantial spatial and temporal variation in damage levels of infestation, adding to the complexity of how the plant and insect species interact with their environment and with each other. In recent studies, models consistently predicted that under various climate change scenarios, disturbance from defoliating insects and foliar pathogens would be detrimental to eucalypt forest primary net productivity (Pinkard et al. 2009b).

With increasing use of growth models in operational management forestry, a proper model validation is a primary concern to achieve simulation process and sustainable management decisions. However, rigorous validation of a model may not be possible because of the lack of suitable data or because the model simulates processes that are not fully understood (Baskerville and Kleinschmidt 1981). Process-based models of plant growth provide a useful framework for determining how leaf removal by defoliating agents influences processes regulating growth. Predictions from the CABALA model (from CARbon BALAnce) have been validated for examining the effects of *Mycosphaerella* leaf diseases (MLD) on rotation-length *E. globulus* plantation productivity under current and future climates, for the range of MLD levels applied (Pinkard et al. 2009a). Moreover, successful application of the CABALA model outputs may depend largely on the choice of appropriate values for the site-specific variables (e.g. soil water-holding capacity), many of which are difficult to measure in the field. The adequacy of site description and sensitivity of model outputs to the resolution and detail of site factors is often discussed and tested (e.g. Mummery and Battaglia 2002). Recent studies have demonstrated that CABALA can be parameterised to simulate tree growth over a wide range of site conditions with useful accuracy (Miehle et al. 2009; Pinkard et al. 2009a), but this success is to some extent a reflection of the availability and reliability of background information on the description of the site. In this study, CABALA was able to predict stem volume (V) of young *E. globulus* trees (Chapter 7) with good accuracy compared to the observed values (Figure 8.1a). However, discrepancies in growth variables (e.g. diameter) and water use (Q) remained (Figure 8.1b, c). Firstly, CABALA accomplishes great predictive

precision as the plantations aged, achieving height model efficiency values at 8 years of age, whereas large prediction errors were reported for young (2-year) *E. globulus* trees (Miehle et al. 2009). Secondly, the adequacy of site description and sensitivity of model outputs the site inputs were a matter to fit the model for the data accurately. By changing some of the site inputs (e.g. increase in soil water-holding capacity), the model then largely over-predicted V of all four treatments, when it was improving the predictions of diameter and water use were improving. There is a need for accurate and reliable site information.

In the process of carrying out the research in this study a number of critical gaps in knowledge limiting the capacity to model the impacts of pest activity on forest productivity have been identified:

- ✓ Insufficient understanding of the levels and frequency of damage that are required to significantly reduce tree NPP under various conditions of stress.
- ✓ Insufficient understanding of distribution and abundance of specific pests, and how they are likely to change in the future.
- ✓ Inadequate knowledge of how, and to what extent, various sources of stress, such as drought, increased temperature, air pollution, and fire, interact to determine tree and stand susceptibility.
- ✓ Paucity of accurate and detailed record on anthropogenic (e.g. land-use) and natural (e.g. history of defoliation) disturbance patterns within sites.
- ✓ Unavailability of long-term data on physiological responses to multiple stressors in old tree stands.
- ✓ Inadequate knowledge of the role played by insects in forest ecosystem function (e.g. nutrient cycling), especially those functions necessary for the long-term sustenance of forest biodiversity and productivity.

Although work in this thesis has shown that quantifying physiological processes and response to defoliation is important in the development of IPM tools; it has highlighted the importance of considering interactive effects of stressors on physiological basis. At present, little information is available. Physiological response to defoliation in interaction with abiotic stressors is crucial to understand. It is necessary to develop

long-term experiments that involve long-term drought conditions associated with high vapour pressure deficit and temperature. Understanding the physiology of defoliation under current and future environment should enable forest managers to identify the likely effects of biological controls that aimed to minimise the detrimental effects of defoliation. In the long term predictive process-based mode of defoliation responses will be of great value to forest managers, but for most species the development of generalised models is still hampered by a lack of detailed physiological information. In a short term, hybrid models may prove useful for some aspect of defoliation management.

Results from this thesis partially validated the four hypotheses proposed in chapter 1 to explain plant response to defoliation. The first hypothesis was tested in the chapter 3, and the results showed that *E. globulus* responds similarly to both artificial and insect defoliations. Nevertheless, some caution must be taken for the extrapolation of results from artificial defoliation in other studies. The second hypothesis on the source:sink relationship was tested throughout the project. It was strongly suggested that *E. globulus* although results showed very short-lived responses. Partial defoliation results in increased photosynthesis per unit leaf area throughout the crown. This was supported by improvement in plant water status following defoliation, which validates the fourth hypothesis. Moreover, *E. globulus* responds to defoliation by adjusting its water status according to the hydraulic model. In both Pittwater experiments, the high resource levels (irrigated) supports *E. globulus* growth and compensation following defoliation. But more interestingly, although the low water supply (rain-fed) may have impaired *E. globulus* capacity for growth compared to the irrigated conditions; the results on compensatory responses to defoliation were unclear. Some discrepancies between the measurements at the leaf and whole tree levels do not completely validate or reject the resource limitation hypothesis. Further studies are required to develop our understating on the interactive effects of stressors.

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